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# ENVIRONMENTAL QUALITY RESEARCH: Fish and Aufwuchs Bioassay Second Annual Report

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## TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

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This report contains the results of research efforts of a project concerned with defining the effects of potential environmental contamination resulting from the use of certain Air Force materials on freshwater and saline water fish. Materials being evaluated include JP-8 and hydrazine. Techniques for exposing organisms to these substances are discussed and results of such exposures are presented.

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## SUMMARY AND CONCLUSIONS

1. This report deals with two topics:

   (i) the toxicity of the jet fuel, JP-8, to the warm fresh water fish golden shiner (Notomigonus chrysoleucas) and flagfish (Jordanella floridae).

   (ii) the toxicity of the rocket fuel, hydrazine, to the estuarine fish species, three-spine stickleback (Gasterosteus aculeatus) and aufwuchs.

2. Gas chromatographic methods have been developed for the qualitative and quantitative analysis of JP-8 in aqueous solutions.

3. The effect of pH on the acute toxicity of JP-8 to golden shiners was examined in static bioassays. Richmond Field Station (RFS) tap water was saturated with the water soluble fraction (WSF) of JP-8 and the solution was renewed each day. Acute toxicity as assessed by the 96-hr LC 50 values was not significantly different over the pH range of 7.3 to 9.1; at pH 7.3 it was 8.5 mg/ℓ (95% confidence limits of 7.65 to 9.52 mg/ℓ) and at pH 9.1 it was 9.6 mg/ℓ (95% confidence limits of 7.85 to 12.23 mg/ℓ).

4. Results of continuous chronic bioassay on the toxicity of the water soluble fraction (WSF) of JP-8 to flagfish supported the following conclusions:

   a. Eggs could not be hatched to viable fry at a WSF of JP-8 concentration of 6.8 ± 1.1 mg/ℓ. There was no significant difference in percent hatchability in the range of 0-3.3 ± o.4 mg/ℓ JP-8, but there was evidence of a delay in hatching for eggs exposed to concentrations ≥ 1.7 mg/ℓ.

   b. Growth and development of flagfish fry as assessed by length measurements was significantly and adversely affected at JP-8 concentrations of 3.3 ± 1.04 mg/ℓ and greater. No effect was discernible in the range 0 - 1.58 ± 0.72 mg/ℓ.

   c. JP-8 concentrations in flagfish whole body tissue reach equilibrium levels after approximately two weeks of exposure. There is a linear relationship between whole body tissue JP-8 concentration and aqueous JP-8 concentration. The ratio of these concentrations is 158.8 ± 33.1 for flagfish whole body tissue. Liver tissue contains equilibrium JP-8 concentrations that are 2.5 times greater than muscle tissue equilibrium JP-8 concentrations.

5. Results of hydrazine studies led to the following conclusions:

   a. The 96-hr LC 50 of hydrazine to three-spine sticklebacks was 3.4 mg/ℓ (nominal initial concentration) using 24 hr solution renewal, but the estimated mean concentration based on the hydrazine degradation rate for a 24 hr period was 2.9 mg/ℓ.

b. In analog tank studies of the effect of a hydrazine "spill," i.e., static bioassay with no hydrazine renewal, the 96-hr LC 50 to sticklebacks was 6.6 mg/ℓ (95% confidence limits of 5.0 to 8.8 mg/ℓ) based on the initial hydrazine concentrations dosed to the tanks. In this concentration range the loss of hydrazine over 96-hr was approximately 50% of initial values as assessed by actual measurements.

c. There was evidence that sticklebacks are not as severely affected by 10 mg/ℓ hydrazine, which is rapidly depleted, as they are by exposure to a more slowly dissipating initial level of 3.2 mg/ℓ hydrazine.

d. Aufwuchs are more sensitive to hydrazine than are sticklebacks. In a "spill" situation an initial hydrazine concentration of 3.2 mg/ℓ (degrading to approximately 1 mg/ℓ in 96-hr) causes complete cessation of metabolic function after 96-hr.

## PREFACE

The research reported herein was conducted at the Sanitary Engineering Research Laboratory, University of California at Berkeley, under the terms of contract F33615-76-C-5005 with the U.S. Air Force. The contract monitor was Lt. Col. Roger C. Inman, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. Professors David Jenkins and Robert C. Cooper were the Principal Investigators. Mr. Stephen Klein was the project manager. Ms. P.C. Ulrichs, Mr. Gary Silverman and Mr. Steven Hergott were responsible for conduct of bioassays. Mr. Steven Krugel, candidate for the M.S. degree in Sanitary Engineering, served as research assistant.

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# 1. INTRODUCTION

Studies included in this report are directed toward providing information on the toxicity of the jet fuel, JP-8, and rocket fuel, hydrazine, on aquatic life. These materials are currently in use by the U.S. Air Force.

To define the environmental impact of a toxicant on aquatic life an experimental protocol has been established by the research project. The protocol includes conducting acute static, acute continuous flow and chronic continuous flow bioassay on warm water and cold water fish species. Studies have been concluded and are reported herein on the effect of the water soluble fraction (WSF) of JP-8 on two warm water fish species, golden shiner (Notemigonus chrysoleucas) and flagfish (Jordanella floridae). The latter species has been studied in chronic bioassays for a six-month period originating with egg hatchability studies followed by a growth and development period and concluding with examination of fish tissue for fuel accumulation. Work yet to be accomplished includes bioassays on cold water fish species and on aufwuchs communities.

Aufwuchs studies will be conducted in saline water at the bay analog facility of the Sanitary Engineering Research Laboratory. This facility was recently refurbished for the primary function of evaluating the toxic effect of hydrazines. However, it will also be ideal for aufwuchs growth as demonstrated in the past by other investigators. Aufwuchs studies in fresh water have not been satisfactory due to lack of proper nutrients.

A static bioassay of the effect of hydrazine on the 3-spine stickleback (Gasterosteus aculeatus) has been completed in the bay analog facility and is reported herein.

# 2. MATERIALS AND METHODS

## FUEL

The jet fuel, JP-8, was supplied by the Aerospace Medical Research Laboratory, USAF. JP-8 is a kerosene-type aviation fuel consisting of a complex mixture of aliphatic and aromatic hydrocarbons. It contains 0.1% of an icing inhibitor, ethylene glycol monomethyl ether, and also has other additives such as metal deactivators and corrosion inhibitors. The specified density of JP-8 is in the range of 0.775 to 0.840 g/ml.

## WATER-SOLUBLE FRACTION (WSF) OF FUEL

For use in static bioassays the water soluble components of JP-8 were prepared by saturating 18 ℓ of a 5% fuel in water mixture over a period of 24 hours in narrow-mouth 5-gal carboys. The air space volume was 2 ℓ and the air-to-liquid volume ratio was 1:9. Each carboy was plugged with a rubber stopper through which a glass sampling tube was placed. The carboys were mixed continuously for 24 hours with magnetic stirrers at a rate which did not create a vortex on the surface and avoided physical dispersion of fuel droplets in the water. Tests indicated that complete saturation could be achieved at this fuel-to-water volumetric ratio and exposure time. After 24 hours the aqueous fuel solution was removed through the sampling device from a point near the bottom of the carboy. The first several hundred ml were discarded. The water was replaced and saturated with the same pool

of fuel to meet the fuel solution requirements for the following day.

For continuous flow bioassays a continuous supply of water saturated with the water soluble fraction of JP-8 was furnished by a fuel contacting device (Figure 1). Two such devices were constructed, each capable of producing 650 mℓ/min of WSF, to meet the 1300 mℓ/min required to operate 10 bioassay tanks in the desired manner.

Each device consists of 5 glass columns (each 1.2m in length and 3.8 cm in diameter) connected in series. The columns each contain 1 ℓ of fuel and the remainder of the liquid column (0.7 ℓ) is water. Water flow through each column of fuel is downward and flow from the final column of the series enters a 9-ℓ capacity glass carboy which serves as a filter and separation chamber. The carboy contains a stainless steel baffle to reduce the terminal velocity of the water stream and a layer of pure fuel to aid in absorbing minutely dispersed fuel droplets.

The tap water entering the device from a constant head tank is first dechlorinated by passage through a 25-gal bed of activated carbon contained in a 50-gal stainless steel drum.

The solubilizers must be cleaned thoroughly on a periodic basis to reduce the bacterial buildup that occurs during operation with JP-8 — a material which can serve as a substrate for microbial growth. During the initial months of operation the schedule for administering a thorough cleaning (using chlorine and soap) was on a monthly basis, and on a weekly basis flushing out with hot water. This was later changed to the use of chlorine and soap on a weekly basis which proved more satisfactory in maintaining flow rates through metering valves and in minimizing changes in JP-8 concentration in the solubilizer product.

The fuel replenishment schedule was replacement of one-half the fuel twice per week during the initial period of operation. This schedule was changed to a once per week replacement of all the fuel to coincide with the cleaning schedule.

## STATIC BIOASSAYS

Static bioassays were conducted in 20-ℓ capacity wide-mouth glass jugs filled to the 15-ℓ mark and provided with the minimum amount of aeration with filtered air to maintain the dissolved oxygen concentration above 4 mg/ℓ throughout the bioassay. The fish weight/dilution water ratio was a minimum of 1ℓ per g of fish as specified in Standard Methods (1970). Fuel volatility loss was ameliorated by daily solution renewals and by covering assay vessels with aluminum foil.

## CONTINUOUS ACUTE BIOASSAYS

Continuous acute bioassays were conducted in a bank of 9 wooden tanks coated with epoxy paint. Each tank had dimensions of 14.9 cm wide, 44.8 cm long and an operating depth of 32 cm to provide a capacity of 21.4 ℓ. The tanks were designed to minimize the surface area in order to reduce loss of fuel by volatilization. The surface area to volume ratio of the tanks was 1:32. The tanks received WSF of JP-8 through the fuel contacting device and delivery system shown in Figure 1.

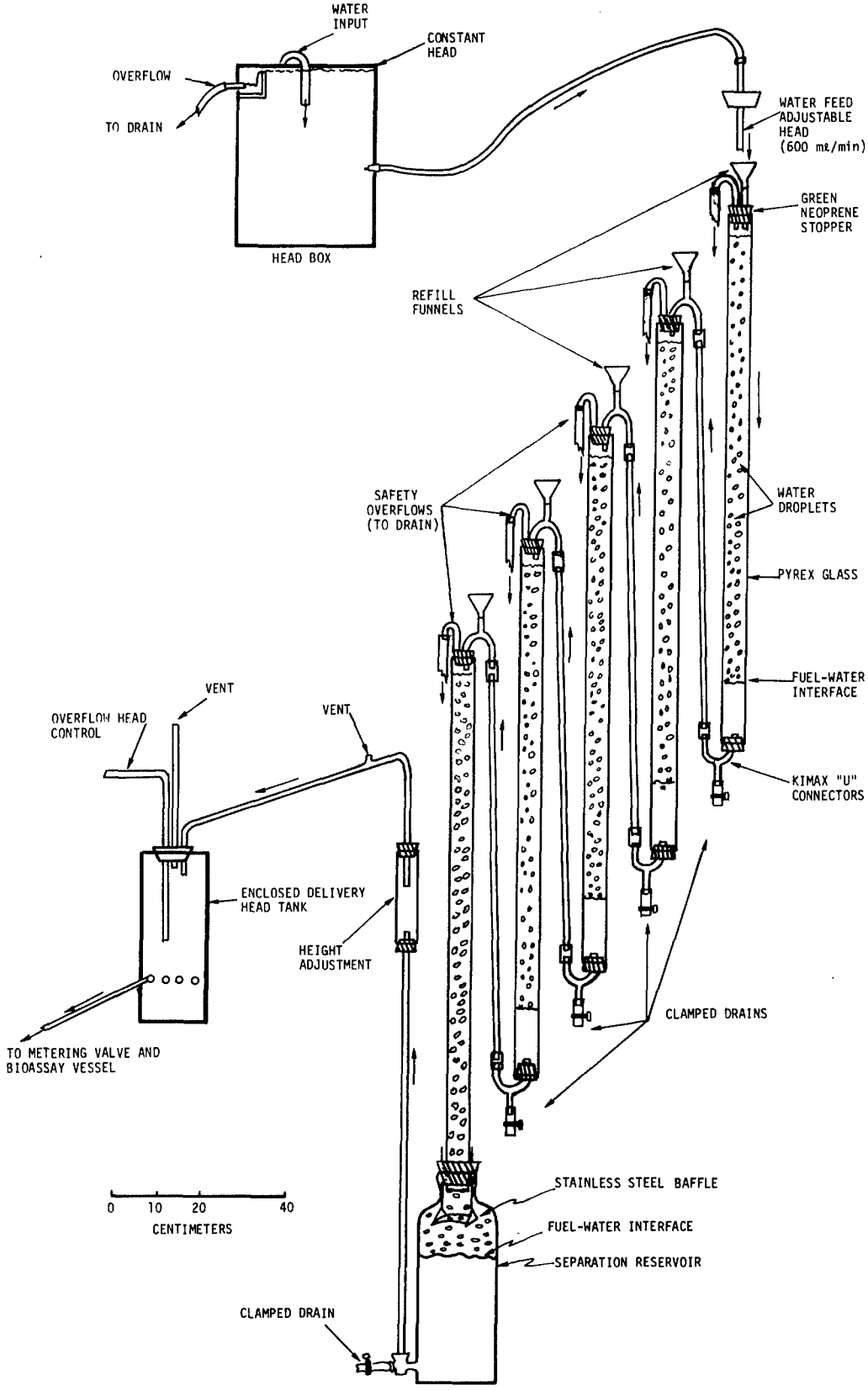



FIGURE 1. FUEL CONTACT AND DELIVERY SYSTEM

## CHRONIC BIOASSAYS

Continuous flow bioassay exposure tanks were of stainless steel construction, 121.9 cm long, 30.5 cm wide and 30.5 cm deep. They could be compartmentalized by removable size 40-010 mesh (0.144 $mm^2$-size opening and 0.25 mm-size wire) stainless steel screens. A standpipe controlled water depth at 8 in (20.3 cm) giving a tank capacity of 80 ℓ. The WSF of JP-8 was produced in the fuel contacting device (Figure 1) and delivered through a manifold system (Figure 2). WSF flow was controlled through stainless steel micro-metering valves located in the manifold 3.2 cm I.D. lucite tube, above each bioassay vessel. Dilution water was fed into a similar manifold at the rate of 1400 mℓ/min and also delivered into bioassay vessels through micro-metering valves.

## EGG CUPS

Egg cups for egg hatchability studies were 4 oz (118.3 cc) glass jars with their bottoms replaced by a 40-010 mesh stainless steel screen. Egg cups were suspended in the stainless steel bioassay vessels and agitated by an electric motor and pulley system as depicted in Figure 3.

## FRY CHAMBERS

Fry chambers were glass, 12 in (30.5 cm) long, 6 in (15.2 cm) wide and 12 in (30.5 cm) deep, with 40-010 mesh stainless screens at each end to permit free circulation of water. The chambers were designed to fit into the stainless steel fish tanks and circulation was provided by the effluent stream from submersible pumps which was directed through the chambers. To reduce volatility loss of fuel caused by prolonged turbulence the pumps were activated by a timer for a period of 1.5 min per hour.

## LC 50 DETERMINATION

LC 50 values were determined by the Standard Methods (1970) technique and by the Reed-Muench method (Woolf, 1968) when 95% confidence limits were computed.

## GOLDEN SHINERS

Golden Shiners were obtained from the Sierra Bait Company, a commercial fish hatchery. Fish were acclimated to dechlorinated Richmond Field Station (RFS) tap water for 1 month before use in bioassays.

## FLAGFISH

Flagfish were obtained from a commercial aquarium and cultured in the laboratory to produce eggs for hatchability and subsequent growth and development studies. These fish are considered to be a species of intermediate sensitivity, and were selected for bioassay work because they are easy to sex, easy to mate and have a short egg-to-egg cycle.

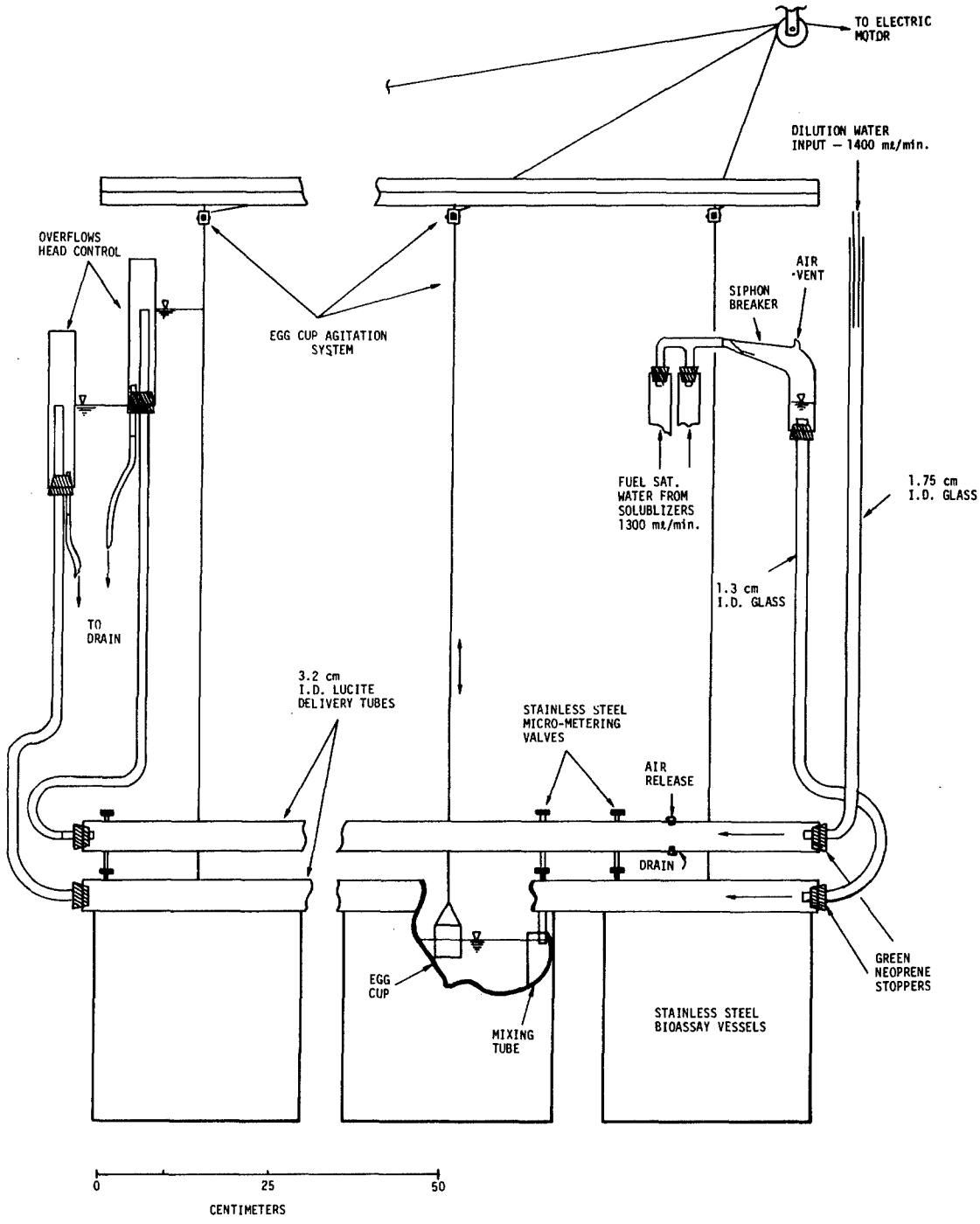



FIGURE 2. CONTINUOUS FLOW BIOASSAY DISTRIBUTION SYSTEM (FRONT VIEW)

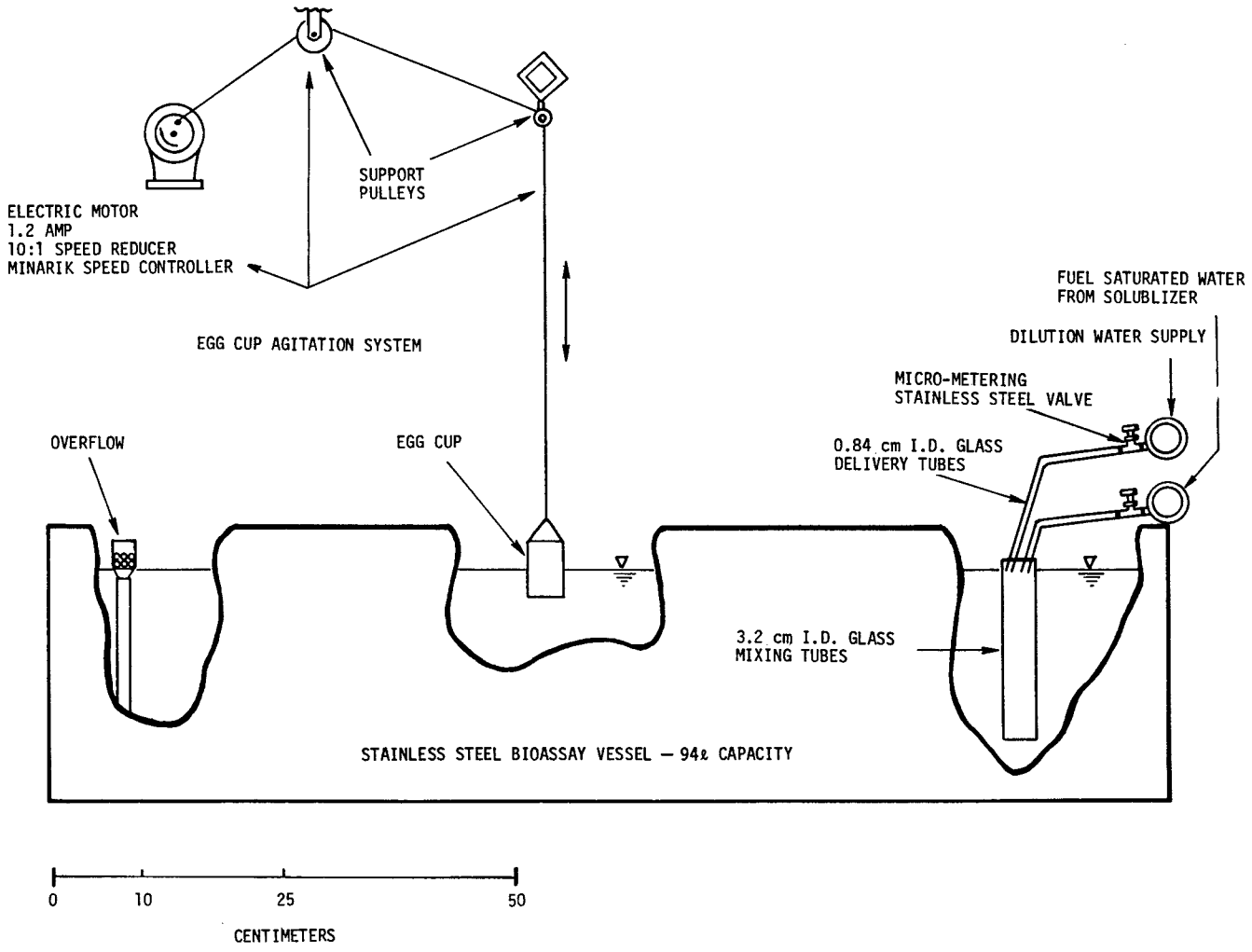



FIGURE 3. SIDE VIEW OF STAINLESS STEEL BIOASSAY VESSEL AND EGG CUP AGITATION SYSTEM

Flagfish spawning aquaria were set up in a separate room from the bioassay room maintained at 22°C, the optimal temperature for breeding. A ratio of one male to 5 females was maintained in 4 spawning aquaria which were 26-gal tanks filtered by commercial pump-filtration systems and aerated by air pumps that dispensed air through air stones. Each tank contained two substrates, consisting of dark green yarn wrapped around stainless steel cloth which received eggs.

Four 15-gal capacity aquaria served as holding tanks for eggs and developing fry. Dechlorinated water flowed through these tanks at a rate of approximately one residence per day and was removed by pumps.

Debris was removed from aquaria daily and the aquaria were thoroughly cleaned once per week by sponging of the sidewalls and bottom. Disease was controlled by malachite green applications.

WATER CHARACTERISTICS

The dilution water was characterized prior to the chronic flagfish bioassay. The water sample was obtained from a point following the continuous flow dechlorinator and before the final solubilizer. The results are presented in Table 1.

Table 1

Water Characteristics

| Parameter | Value |
|---|---|
| total solids | 64 mg/ℓ |
| total dissolved solids | 76 mg/ℓ |
| total hardness (as $CaCO_3$) | 50 mg/ℓ |
| $Cl^-$ | 4.9 mg/ℓ |
| $Mg^{++}$ | 3.0 mg/ℓ |
| $Ca^{++}$ | 11.0 mg/ℓ |
| $Na^+$ | 6.5 mg/ℓ |
| $K^+$ | 0.5 mg/ℓ |
| $SO_4^=$ | 2.0 mg/ℓ |
| pH | 8.9 |
| conductivity | 9.2 micromhos |

## EXPRESSION OF FUEL CONCENTRATION

For static bioassays fuel concentration was expressed in two ways: volumetrically as 100% (saturated) or some dilution of the 100% solution, and gravimetrically (mg/ℓ) by GC analysis of the 100% fuel concentration. The gravimetric concentration of dilutions other than 100% was computed by a direct ratio of the dilution to 100% and multiplying this ratio by the mg/ℓ value for the 100% solution. For continuous flow bioassays the concentrations of all dilutions were measured gravimetrically by GC analysis on a weekly basis to verify the micro-metering valve settings and to account for volatility loss.

## GAS CHROMATOGRAPHIC (GC) ANALYSIS OF FUELS

Aqueous fuel concentrations were determined by GC analysis using a Fisher Model 4800 gas chromatograph with dual flame ionization detectors and 6.1 m x 0.32 cm o.d. stainless steel columns of 10% SE30 on 80/100 Chrom W. Accessory GC equipment included a Fisher Series 5000 Recordall recorder and an Autolab minigrator for digital integration of peaks.

For determination of changes in the fuel composition, it was necessary to use temperature programming, but for reliable quantitation of total fuel concentration, isothermal operation was better. Temperature changes apparently produced electrical noise, resulting in erratic results during programming.

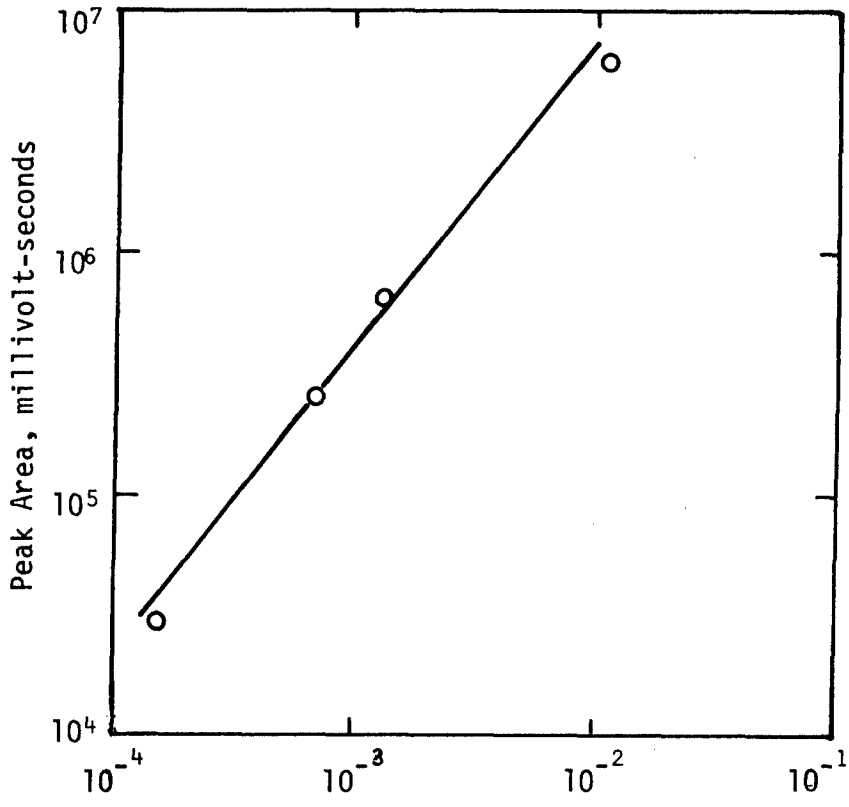
Temperature Program Operation. For JP-8 the most suitable program for peak differentiation was: 60°C for 10 min: increase to 180°C at 4°C/min; final delay at 180°C for 20 min.

Isothermal Operation. A temperature of 160°C was used for quantitative analysis of JP-8. For both isothermal and temperature-programmed operation, the injection and detector temperatures were 270°C and the $N_2$ carrier gas flow rotameter setting was 3.0 at 80 psig. The detector flame $H_2$ flow was set at a rotameter reading of 4.5.

Standard Curve. A standard curve for the GC analysis of JP-8 indicated linearity in the range of $10^{-2}$ to $10^{-4}$g(Figure 4).

Chromatograms of JP-8 (Figures 5 and 6) show that temperature programming provides peak separation and is necessary for evaluating qualitative changes in the fuel composition. Under isothermal operation at 160°C, peaks in the $C_6$-$C_{10}$ range elute rapidly and immediately after the solvent peaks.

Internal Standard. The internal standard selected for JP-8 was n-pentadecane, $C_{15}$, which elutes after all of the JP-8 components. The relationship between JP-8 mass and mass of $C_{15}$ is:




| GC Conditions |
|---|
| Dual Column Operation |
| Temperature Programming |
| Injector Temperature 270° C |
| Detector Temperature 270° C |
| Injection Volume 1.0 µℓ |

FIGURE 4. STANDARD CURVE FOR JP-8

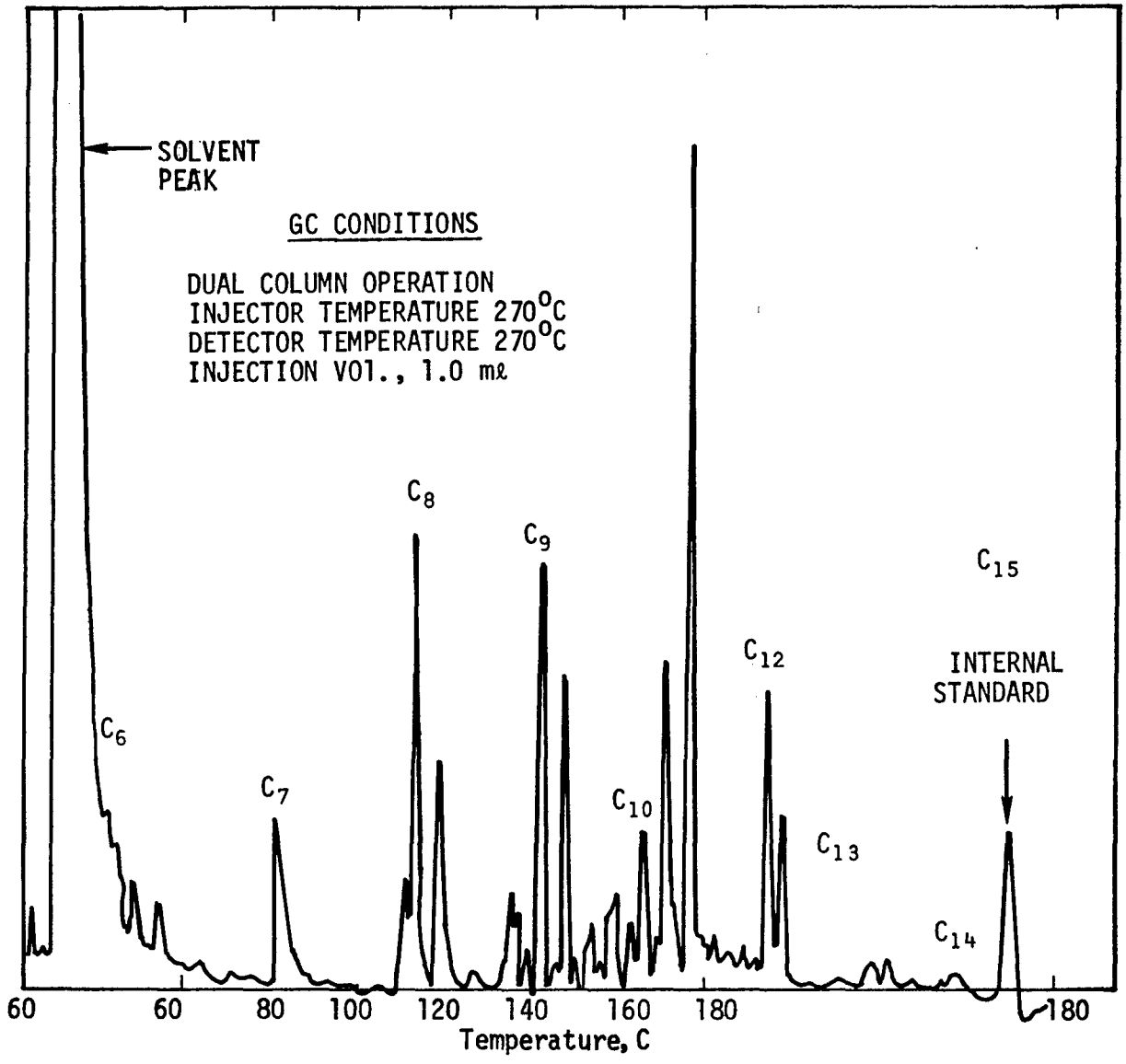



FIGURE 5. CHROMATOGRAM OF JP-8 USING TEMPERATURE PROGRAMMING

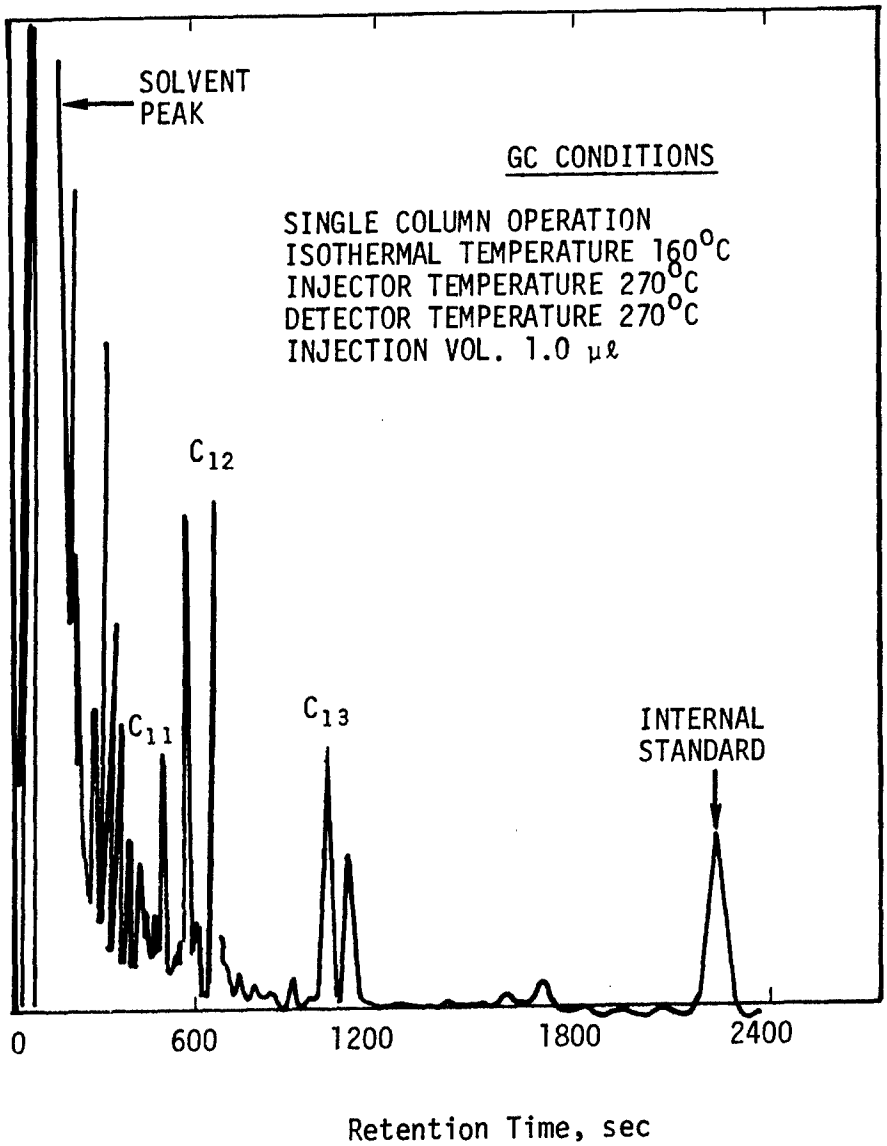



FIGURE 6. CHROMATOGRAM OF JP-8 USING ISOTHERMAL OPERATION

$$M_{JP-8} = M_{C_{15}} \frac{A_{JP-8}}{A_{C_{15}}} . K$$

where $M_{JP-8}$ = mass of JP-8

$M_{C_{15}}$ = mass of $C_{15}$

$A_{JP-8}$ = area of JP-8

$A_{C_{15}}$ = area of $C_{15}$

K = constant

The constant, K, was determined by injecting a series of known masses of JP-8 and $C_{15}$ and measuring their respective peak areas using isothermal operation. The value of K was found to be 1.0.

Although JP-8 contains a $C_{15}$ component, the insolubility of $C_{15}$ in water prevents its interference in the above relationship when applied to the water soluble fraction (WSF) of JP-8.

GC Reproducibility. A series of 7 repeated injections of the same sample of JP-8 indicated a coefficient of variation of 1.77% (using the internal standard method which eliminates error due to variation in injection volumes).

Extraction of Fuel from Water.

The following method was developed for extracting fuel from water:

a. To 300 mℓ sample, add 25 mℓ nanograde n-pentane and shake 2 min by movement of the separatory funnel through a 90° arc at a rate of one complete downward and upward movement per sec.

b. If necessary to avoid emulsions, add 50 mℓ salt solution (100 g NaCl/ℓ distilled water) to original sample.

c. Allow to separate for approximately 10 min, remove aqueous layer, and dewater the pentane layer by passing it through a 3-cm diameter by 3.5-cm deep column of anhydrous sodium sulfate.

d. Repeat steps a and c and combine extracts.

e. Concentrate the combined extract on a rotary evaporator to between 10-15 mℓ.

f. Transfer to 25-mℓ capacity graduated concentrator tube and evaporate to 3.0 mℓ by directing a stream of $N_2$ at the pentane surface.

g. Perform GC analysis.

JP-8 Evaporation Correction

In the process of evaporating the pentane extract of JP-8 down to 3.0 mℓ a portion of the JP-8 present is also lost. The magnitude of this loss was determined by GC injection of the pentane extract before evaporation, and comparing the result with that obtained after evaporation. For 10 samples of the WSF of JP-8 taken from the solubilizer the "before evaporation" results averaged 11.65 ±0.85 mg/ℓ and the "after evaporation" results averaged 7.75 ± 0.40 mg/ℓ. The mean % loss based on the "before evaporation" value was 33.29 ± 4.57% with a coefficient of variation $C_v$, of 13.73%.

For computation purposes a correction factor of 1.5032 was used to multiply the results obtained for evaporated samples.

In order to obtain adequate GC sensitivity in the analysis of "before evaporation" samples, very large injections (on the order of 8 μℓ) were required. Samples could not be processed routinely in this manner because repeated injections of this volume caused GC operational problems.

GC Analytical Precision

The precision of the JP-8 analytical method based on 69 pairs of duplicate analyses was 0.24 mg/ℓ (Appendix A), as assessed by the standard deviation of the 69 individual standard deviations. This computation neglects the loss of precision due to the periodic need to prepare and dilute fresh internal standard solutions, and the possible effect of storage on stock internal standard solutions. The latter effect was minimized by storage at -20°C in vials with polyseal caps sealed with parafilm.

Solubilizer Product JP-8 Concentration

Fluctuations in solubilizer product JP-8 concentration from day to day was a function not only of the degree of analytical precision, but also of fuel biodegradation, frequency of apparatus cleaning to minimize biodegradation and frequency of fuel replenishment. Figure 7 demonstrates the effect of fuel age on JP-8 concentration. During the period that these samples were taken the routine procedure was to clean the apparatus and replenish the fuel at weekly intervals. For the samples taken 18 hrs after cleaning the mean JP-8 concentration was 12.44 ± 0.715 mg/ℓ, and for the samples taken 164 hours after cleaning the mean JP-8 concentration was 10.67 ± 0.705 mg/ℓ. To what extent this decrease of 1.77 mg/ℓ is due to biodegradation or to physical depletion of soluble components is not known. Although there was a general depletion of JP-8 components, detailed examination of chromatographs did not conclusively show differential depletion of individual components.

The mean JP-8 concentration of the solubilizer product over a 152-day period of operation was 10.98 mg/ℓ ± 0.94 mg/ℓ (see Appendix A).

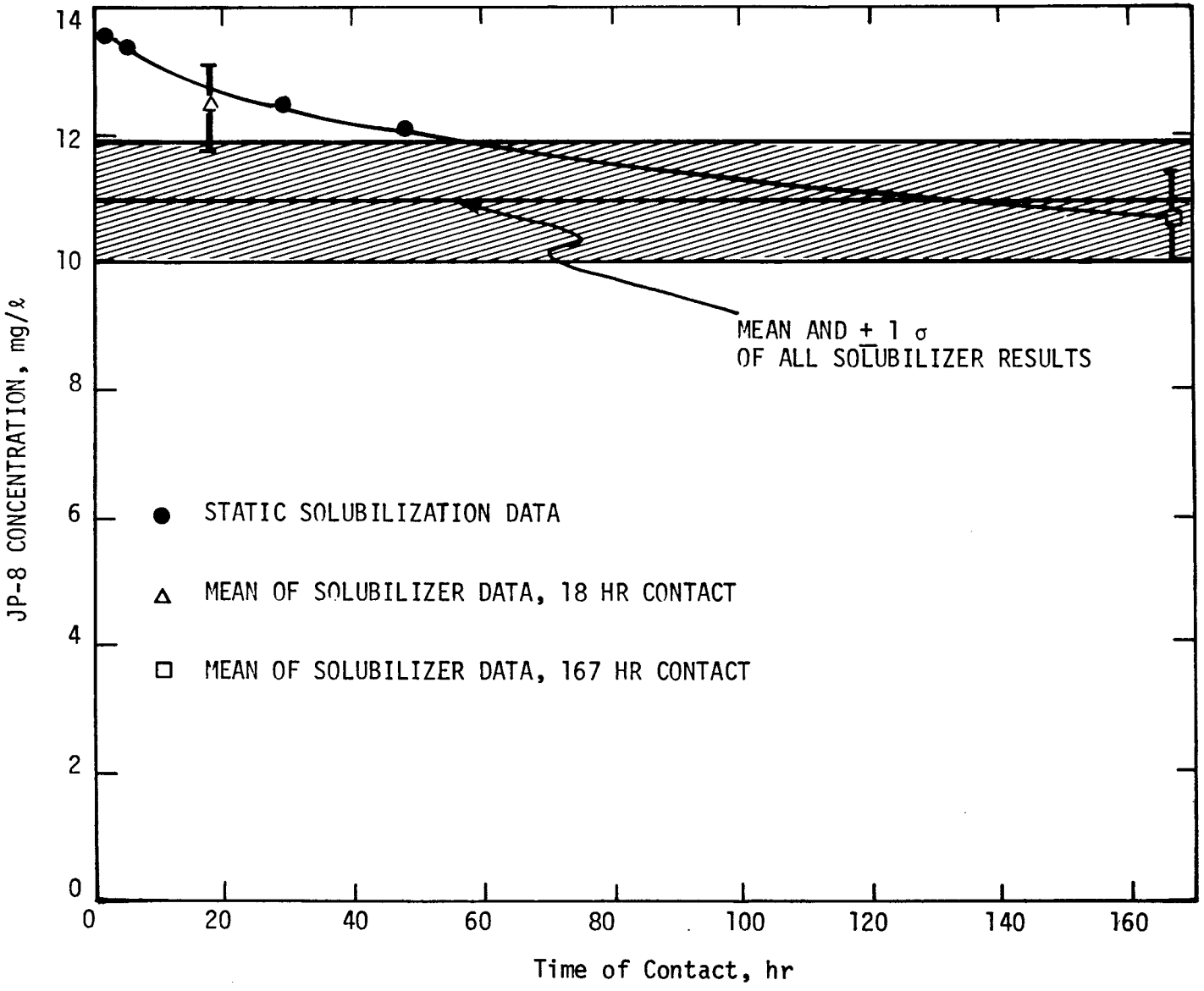



FIGURE 7. SOLUBILITY OF JP-8 IN WATER USING STATIC AND CONTINUOUS SOLUBILIZATION

## EXTRACTION OF JP-8 FROM FISH

Fuel accumulation in the whole body tissue and the muscle tissue was determined by grinding fish in a Virtis "45" tissue grinder in the presence of pentane and sodium sulfate. Liver tissue was ground in a glass tissue grinder in the same manner. Particulate matter and water were removed by filtering the pentane extract through sodium sulfate, followed by concentration to 15-25 mℓ using a rotary evaporater, filtration through florosil, and volume reduction by rotary evaporation to the desired volume (usually 3 mℓ ) for GC analysis.

## CENTRAL BAY ANALOG TANK OPERATIONS

Studies of the effect of hydrazine on 3-spine sticklebacks (Gasterosteus aculeatus) and aufwuchs were conducted in San Francisco Bay water pumped from the central San Francisco Bay to analog tanks located at SERL. The 12 Central Bay model tanks were rectangular in shape and each of 4.2 cubic meter capacity (3 m long, 1.22 m wide and 1.14 m deep). The tanks were of heavily reinforced marine plywood construction with all surfaces, interior and exterior fiberglassed.

In each tank there was an air agitation system consisting of 2.5 cm PVC pipe perforated along its entire 1-m length and connected with flexible tubing to a compressed air header. The air mixer was mounted 30 cm above the bottom and 30 cm out from the tank wall along the short axis of the tank.

Bay water pumped to the tanks entered plexiglas 20-ℓ capacity constant head boxes. One such box served each cluster of 4 tanks (see Figure 8). Water flow to each tank was regulated by 1.3 cm PVC valves.

Discharge from the analog tanks was piped to a treatment tank for hydrazine oxidation. The tank, 8 ft. (244 cm) and 4 ft (122 cm) x 2 ft (61 cm) deep was constructed of epoxy-coated plywood and was baffled as shown in Figure 8, to prevent short circuiting. Hydrazine was oxidized by chlorine added through a drip bottle in the form of 5.25% sodium hypochlorite solution.

## CENTRAL SAN FRANCISCO BY WATER

Bay water was delivered to the analog tanks from an intake 3400 ft (1.04 km) offshore. This system delivered good quality Bay water representative of that in the Central San Francisco Bay. The intake location was sufficiently deep to remain well-submerged at low tide and permit continuous operation. A sketch of the salt water supply system is shown in Figure 9. The intake station consisted of a 7.6 cm flexible polyethylene pipe anchored to a platform piling at a point 0.3 m above the bay bottom. Water was withdrawn by a 5 hp 1750 RPM Gould pump located 3000 ft (0.91 km) from the intake at the end of a 400 ft pier. The pumping unit was in a protective casing residing on the bottom of the slough to minimize head loss from the intake point. Priming was accomplished by two pumps which withdrew slough water and backflushed the pipe line for 2 hrs before suction was initiated by the main pump. The bay water was delivered to the analog tanks which were located approximately 488 m inland from the pump station.

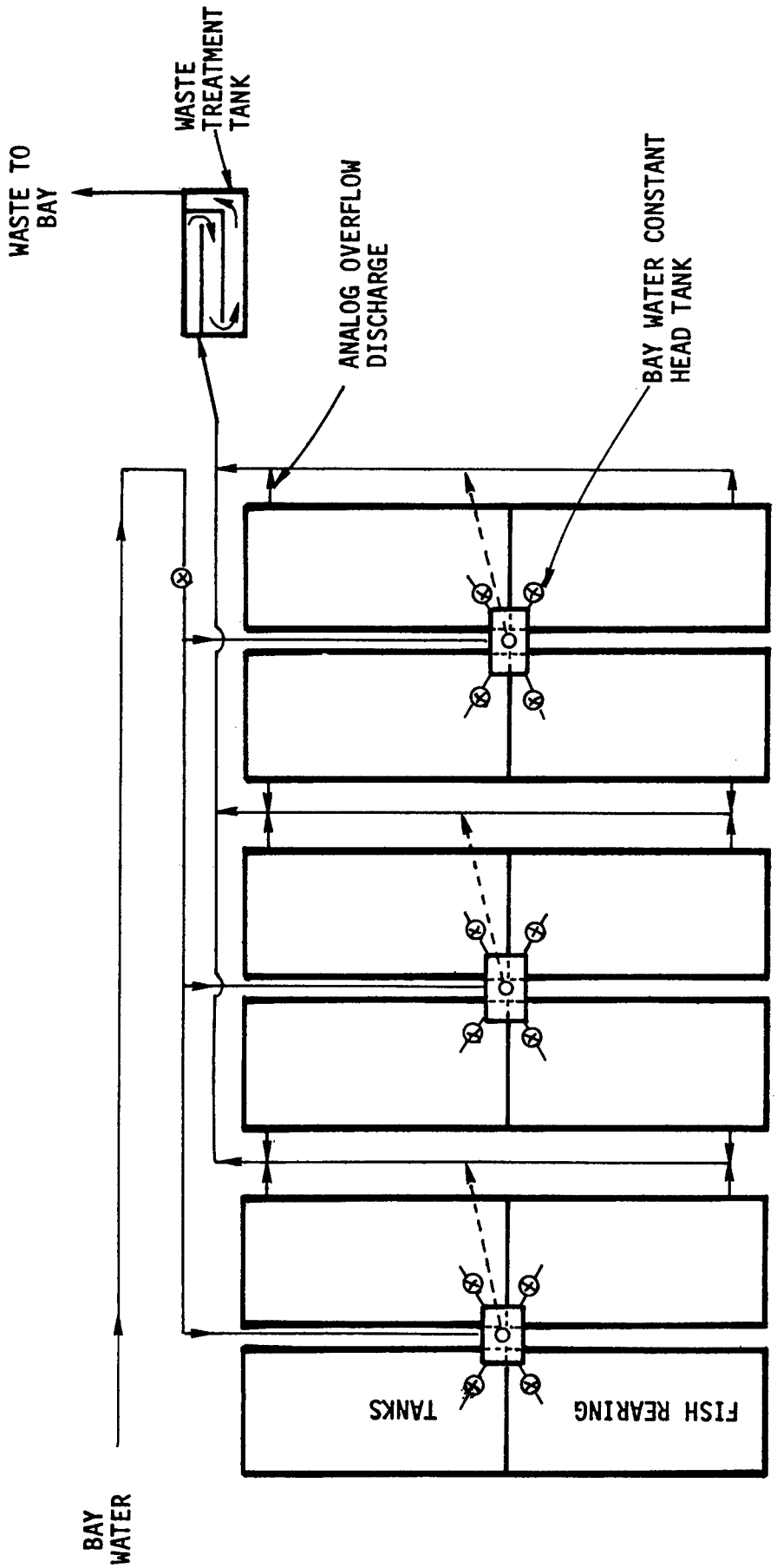



FIGURE 8. ANALOG SYSTEM

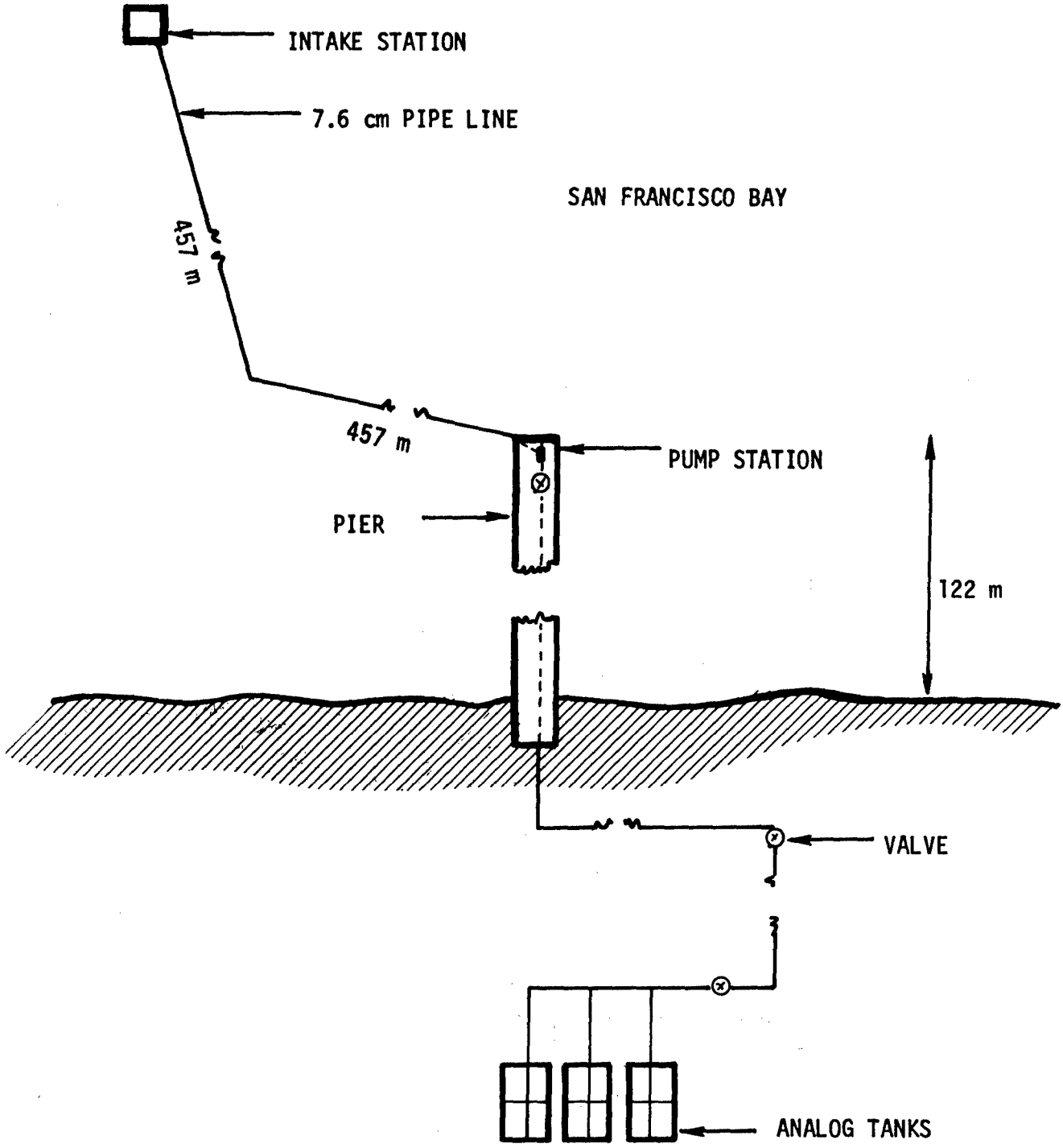



FIGURE 9. ANALOG SYSTEM SALT WATER SUPPLY LINE

## SALINE WATER CHARACTERISTICS

In the analog static bioassay of hydrazine toxicity to fish and aufwuchs the water characteristics were examined by removing water from one of the analogs and determining chlorosity, total alkalinity, suspended solids, $NH_3$-N, COD and pH. All determinations were performed in accordance with Standard Methods (1975) except for $NH_3$-N analysis which was determined by the Phenol-hypochlorite Method (Solarzano, 1969). The results indicate a chlorosity of 18.0 g/ℓ, total alkalinity of 13.3 mg/ℓ suspended solids of 0.021 mg/ℓ, $NH_3$-N of 0.09 mg/ℓ, COD of 1800 mg/ℓ and pH of 7.85.

## FISH

The fish species examined for sensitivity to hydrazine toxicity was the three-spine stickleback (Gasterosteus aculeatus). This fish is native to the water of Central San Francisco Bay and a commonly-used indicator species in saline water bioassays.

## FISH CAGES

Cylindrical cages, open at the top, contained fish during analog tank studies. Fish were isolated for ease of monitoring and to protect aufwuchs. The cages, 30.5 cm in diameter and 38 cm in height, were constructed of a 1.3 cm PVC pipe framework supporting 18 x 16 mesh fiberglass netting. The top 10 cm of the cages was kept above the water surface to prevent fish from escaping and to facilitate removal of dead fish.

## HYDRAZINE

Hydrazine monohydrate of 95+ % purity was obtained from Eastman Organic Chemicals Division of the Eastman Kodak Company.

## HYDRAZINE ANALYSIS

Hydrazine was analyzed by the method of Watt and Chrisp (1952) which is based on the development of a yellow color upon the addition of p-dimethyl-aminobenzaldehyde (DMBA) to solutions of hydrazine in dilute HCl. The percent transmittance at 458 nm was read on a Beckman Model DU Spectrophotometer. The method has a sensitivity of 0.01 mg/ℓ.

## HYDRAZINE DECAY IN SALT WATER

The rate of hydrazine decay was determined by preparing a solution of 10 mg/ℓ hydrazine in bay water and withdrawing samples at intervals during an 8-day period. Results for time intervals of 5, 15, 30, 60, 120, 1440 and 11520 minutes are H concentrations of 10.6, 10.6, 10.1, 9.9, 9.9, 9.9, 8.1, and 0.9 mg/ℓ respectively.

## AUFWUCHS

Each analog tank contained one growth rack suspended 50 cm beneath the water surface. Each rack accommodated 30 growth units consisting of roughened tygon tubing, 5 cm long and 1.2 cm in diameter.

AUFWUCHS EXAMINATION TECHNIQUES

Eight growth units were removed from each of the 7 analog tanks at the following times:

(i) Prior to hydrazine addition (to establish variation between tanks).
(ii) Two days after hydrazine addition.
(iii) Four days after hydrazine addition.

Growth units were examined for metabolic response, chlorophyll a content and biomass.

Metabolic response (photosynthesis and respiration) was assessed by a light/dark bottle technique. Four of the aufwuchs were each placed in four opaque BOD bottles, and four aufwuchs were placed in clear BOD bottles. The BOD bottles contained water taken from the same tank that the aufwuchs came from. Two more BOD bottles contained water only and on these DO was measured with a Yellow Springs Instrument Model 57 Oxygen Meter with a Model 5420A Self-stirring Bottle Probe. All ten bottles were placed in a 15C water bath and illuminated at approximately 1500 foot candles with cool fluorescent lights. After 2 hrs the translucent bottles were removed and their DO determined. After 4 hours the opaque bottles were removed and their DO was similarly determined.

Metabolic response to the light and dark treatment was calculated following Standard Methods (1975) page 1039 as follows:

Net photosynthesis = light bottle DO - initial DO
Respiration = initial DO - dark bottle DO
Gross Photosynthesis = light bottle $DO/_{hr}$ - dark bottle $DO/_{hr}$

$$\text{Photosynthetic Index} = \frac{\text{Gross Photosynthesis, mg } O_2}{\text{Organic mass, g dry wt.}}$$

For biomass and chlorophyll a analyses the growth units were removed from BOD bottles, and the growth separated from the substrate by passing the tygon tubing through a rubber stopper holed to the same diameter as the tubing. Aufwuchs growth that had separated while in the BOD bottle was recovered by filtering the bottle contents through a tared glass fiber filter. The filter, also containing the growth from the tygon tubing, was placed in a plastic bottle. An aqueous solution of 90% acetone by volume was added to the plastic bottle and stored at 4C overnight. The filter was then removed from the bottle with forceps, placed on the filtration apparatus, and the bottle contents filtered through it. The filtrate was analyzed for Chlorophyll a by the Trichomatic Method, page 1030 Standard Methods (1975). On occasion, when immediate chlorophyll analysis was not possible, the filtrates were preserved by adding 0.05 mℓ of a 10 g/ℓ $MgCl_2$ solution prior to filtration, then freezing the filtrate until the determination could be made. The filter was saved for biomass determinations.

Table 2

Experimental Conditions of High pH Study

| Soluble JP-8 Volume | pH | | | | | | Dissolved Oxygen, mg/ℓ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time, hr. | | | | | | Time, hr. | | | | | | | |
| | 24 | | 24 | | 24 | | 24 | | 24 | | 24 | | 24 | |
| % | i* | f** | i | f | i | f | i | f | i | f | i | f | i | f |
| 100 | 8.6 | 7.2 | 8.9 | | 8.9 | 7.2 | 7.6 | 5.0 | 7.7 | 5.9 | 7.7 | 6.2 | 7.7 | 6.2 |
| 79 | 8.8 | 7.3 | | | | 7.2 | | 5.1 | | 4.9 | | 4.8 | | |
| 50 | 8.8 | 7.3 | | | | 7.3 | | 5.5 | | 5.1 | | 5.0 | | |
| 32 | 9.0 | 7.3 | | | | 7.3 | | 5.5 | | 5.1 | | 5.2 | | |
| 20 | 9.1 | 7.3 | | | | 7.3 | | 6.4 | | 6.1 | | 5.2 | | |
| 0 | 9.1 | 7.3 | 9.1 | | 9.1 | 7.3 | 7.8 | 6.3 | 7.9 | 6.1 | | 5.9 | 7.8 | 5.0 |

* initial
** final

Table 3

Survival of Golden Shiners Exposed to Soluble JP-8

| Soluble JP-8 Conc. Volume | | Fish Survival, Number Time, hr. | | | |
|---|---|---|---|---|---|
| % | mg/ℓ | 24 | 48 | 72 | 96 |
| 100 | 12.1 | 8 | 7 | 4 | 2 |
| 79 | 9.6 | 10 | 10 | 10 | 5 |
| 50 | 6.1 | 10 | 9 | 9 | 9 |
| 32 | 3.9 | 10 | 10 | 10 | 10 |
| 20 | 2.4 | 10 | 10 | 10 | 10 |
| 0 | 0.0 | 10 | 10 | 10 | 10 |

Biomass determinations were gravimetric using the Standard Methods (1975) techniques for plankton biomass estimation.

## 3. STATIC BIOASSAY-JP-8

### INTRODUCTION

Static bioassays were conducted on golden shiners exposed to the WSF of JP-8 to investigate the effect of pH on toxicity. Previous static bioassays have shown that water hardness does not affect JP-8 toxicity to golden shiners (Annual Report, 1976). In those studies the dilution water pH was 7.3 to 7.6 and the results obtained at that pH range may be compared to results of a more recent study conducted with dilution water having a pH of 8.6 to 9.1.

The reason for the difference in pH between the two studies was caused by the manner of dechlorination. In the first study dilution water was subjected to a 24-hr pre-aeration period which lowered the pH prior to initiation of the bioassay. The latter study received no pre-aeration, but was dechlorinated by a carbon filter.

### PROCEDURE

The dilution water was dechlorinated RFS tap water obtained from the overflow of the continuous flow system after passage through the carbon filter for dechlorination. The water was then saturated with the soluble components of JP-8 in the manner previously described for static bioassays, and the assays were performed as described in the Materials and Methods Section.

The test species, golden shiners, had an average standard length of 4.58 cm $\pm$ 0.06 cm and an average wet weight of 1.36g. The temperature was maintained at 22$^{o}$C, vessels received gentle aeration from the house air supply, and solutions were renewed daily to minimize fuel volatility loss during the 96-hr test period. No pH adjustment was made to the water which was used as received from the dechlorinator.

Golden shiners were exposed to six serial dilutions of the WSF of JP-8 ranging from 0 to 100%.

### RESULTS

The pH results (Table 2) indicate that the initial pH each day was between 8.6 and 9.1 and the final pH each day decreased to approximately 7.3 due to the gentle aeration received over the 24-hr period and to fish respiration. The dissolved oxygen results indicated no problem with maintenance of a sufficient D.O. throughout the test period.

The fish survival data (Table 3) indicated that the 96-hr LC 50 was at the 79% volumetric concentrations (equivalent to 9.6 mg/$\ell$ JP-8 based on 100% JP-8 = 12.1 mg/$\ell$ $\pm$ 1.162 mg/$\ell$). The LC 50 based on the Reed-Muench method (Woolf, 1968) was 9.81 with 95% confidence limits of 7.85 to 12.23 mg/$\ell$.

DISCUSSION

The results of the previously reported bioassay in the lower pH range had indicated a 96-hr LC 50 of 70% on a volumetric basis (equivalent to 8.5 mg/ℓ JP-8). The LC 50 based on the Reed-Muench method was 8.5 with 95% confidence limits of 7.65 to 9.52 mg/ℓ. Therefore, the results of the current study were not significantly different from the previous study and it may be concluded that pH in the range of 7.3 to 9.1 does not significantly affect the toxicity of the WSF of JP-8 to golden shiners.

## 4. CONTINUOUS ACUTE BIOASSAY-JP-8

A short-term continuous acute bioassay was conducted for range-finding purposes in order to establish the dilutions of the WSF of JP-8 to be used subsequently in a long-term chronic bioassay with flagfish.

The assay was conducted in the bank of 9 epoxy-coated wooden tanks described previously. A continuous supply of the WSF of JP-8 was furnished to each assay tank from an enclosed head tank which received the solubilizer product (see Figure 1). Flows of WSF and dilution water were regulated by metering valves to each tank to provide the following nominal percentages of WSF of JP-8: 100, 79, 50, 32, 20 and 0.

The flagfish used had an average standard length of 2.47 cm $\pm$ 0.03 cm (standard error of the mean) and an average wet weight of 0.50 g. Ten fish were placed in each tank of 21ℓ water volume, and the tanks were operated at a nominal retention time of 2 hr. The source of dilution water and solubilizer water was RFS tap water dechlorinated by passage through a charcoal filter. The temperature was measured daily and was maintained at 24.3 $\pm$ 0.8 $^{0}$C.

No aeration was provided, and weekly measurements indicated that the dissolved oxygen remained at a satisfactory level of 7.12 $\pm$ 0.1 in the control and 7.13 $\pm$ 0.15 in the 100% tank. The pH, measured weekly, remained virtually constant in all tanks at 9.0 $\pm$ 0.1.

The fish survival data (Table 4) indicate 100% survival in all tanks through day 13. Mortality then occurred in the 100% tank followed later by mortality in the 79% tank. There was 100% survival in all other tanks throughout the 21-day duration of the experiment. Thus, the LC 50 for 15 days was 100% WSF of JP-8 or 10.8 mg/ℓ JP-8 and the 21-day LC 50 was 62% or 6.7 mg/ℓ JP-8.

The sub-lethal behavioral characteristics of flagfish were similar to those stress symptoms exhibited by golden shiners. Within the first 5 min after exposure to tanks containing fuel all flagfish were swimming at the surface, while the control fish swam at the bottom. Subsequently, only the fish in the 100% tank remained at the surface.

After 24-hr, fish in the 100% tank and 79% tank had a dark coloration. After 48-hr all fish in the 100% tank were moribund; all fish in the 79% tank were again at the surface and 8 were black. There was a slight improvement

Table 4

Survival of Flagfish Exposed to the WSF of JP-8

| JP-8 Concentration | | | Fish Survival, Number | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Time, days | | | | | | | | |
| % Desired WSF | % Measured WSF ± s | mg/ℓ | 0-13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| 100 | 100 | 10.8 | 10 | 8 | 5 | 3 | 1 | 1 | 0 | 0 | 0 |
| 79 | 78 ± 4 | 8.4 | 10 | 10 | 10 | 9 | 9 | 8 | 6 | 2 | 1 |
| 50 | 47 ± 5 | 5.1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 32 | 31 ± 3 | 3.3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 20 | 19 ± 3 | 2.1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 0 | 0 | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Note:

The measured percent WSF is based on daily measurements of the solubilizer and dilution water inputs to each tank. The mean JP-8 concentration of 10.8 mg/ℓ is the average of 11 GC measurements taken from the 100% tank, and the sample standard deviation, s, of these measurements was 0.7 mg/ℓ.

in some of the moribund fish during the next 48 hr, but all fish in the concentrations of 50% swam at the surface and showed a darker than normal coloration. Even at the lower concentrations slight stress symptoms were observed — the test fish did not appear as active as the control fish. These behavioral symptoms persisted throughout the experiment.

The eating behavior of the fish was also indicative of stress. Live brine shrimp were fed to fish on Monday, Wednesday and Friday of each week. After 24 hr the fish in the concentrations above 20% did not eat; the fish at 20% were slow to respond but eventually ate. By contrast, the control fish ate enthusiastically.

After 72 hr fish in the 100% and 79% tanks did not eat; those in the 50% tank had difficulty in eating and those in the 32% tank ate lackadaisically. In the 20% and control tanks the eating habits were normal. This pattern of behavior remained much the same throughout the experiment.

The results of this study indicated that the no effect level in chronic studies would be expected to be less than 3 mg/ℓ JP-8, and that the dilution series in chronic studies should be planned accordingly.

## 5. CONTINUOUS CHRONIC BIOASSAY-JP-8

### INTRODUCTION

A chronic long-term bioassay was conducted to determine the LC 50 and the no effect level of water soluble JP-8 to flagfish. The no effect level was assessed by egg hatchability studies, duplicate fry growth and development studies and measurement of the accumulation of fuel in body tissue.

### PROCEDURE

Five duplicate concentrations of fuel were used in 10 stainless steel tanks. Based on the results of the continuous acute study, the percentages of the WSF of JP-8 selected were 75, 42, 24, 14 and 0. The tanks were operated at a depth of 20.3 cm yielding a volume of 80 ℓ. The total flow to each tank was 220 mℓ/min giving a theoretical residence time of 6 hr. To provide the desired percentages of fuel the flow of fuel and dilution water to each tank is shown in Table 5.

The two banks of 5 tanks each received the WSF of JP-8 from the solubulizer. A water flow of 600 mℓ/min was initially fed into each solubilizer, and later in the experiment the flow was reduced to 450 mℓ/min. The total flow of 900 mℓ/min provided sufficient WSF of JP-8 for experimental purposes and avoided fuel displacement by water in the solubilizer which occasionally occurred at the higher flow rate.

### RESULTS OF EGG HATCHABILITY STUDIES

Before a successful hatchability study could be conducted, several severe problems had to be resolved. In initial studies there was a repeated loss of large numbers of eggs due to fungal growth. This problem was overcome by rigid adherence to a practice of daily transfer of eggs into clean cups. Although the early bioassays were unsuitable for defining the effect of fuel

Table 5.

Fuel and Dilution Water Flow Rates

| Tank No | JP-8 (WSF) mℓ/min | Water mℓ/min |
|---|---|---|
| 1 | 0 | 220 |
| 2 | 31 | 189 |
| 3 | 52 | 168 |
| 4 | 92 | 128 |
| 5 | 189 | 31 |

on hatchability, the fry produced from these studies were utilized later in the growth and development phase of the chronic bioassay.

Early work was also useful in showing that no viable fry could be hatched at the 75% toxicant level (equivalent to 8.1 mg/ℓ JP-8), but that eggs exposed to this level of fuel for one week could be hatched to viable fry if transferred to fuel-free water. Results of these early experiments also suggested that increasing levels of toxicant increased the time required for hatching. Most important, these early studies indicated the need to compensate for fuel volatility loss in the exposure tanks by increasing slightly the percentage concentration delivered. Thus the percentages of WSF of JP-8 increased from 14, 24, 42 and 75 to 16, 29, 48 and 86%, respectively, for tanks 2, 3, 4 and 5, while maintaining the control tank, 1 at 0% fuel.

In the major egg hatchability study, each of 5 tanks received 270 eggs. The 270 eggs were placed into two different egg cups to avoid overcrowding and to keep the age differences between eggs to a minimum. Thus each egg cup received 100 1-2 day old eggs, then two days later 170, 1-2 day old eggs were added to a second egg cup. Eggs were examined, and transferred daily into clean cups. As the eggs hatched, the fry were transferred into a third egg cup. Used cups were treated by submerging them in boiling water for 1 hour.

Fry hatchability results are presented in Figure 10. In the upper graph % fry hatched is plotted vs. time where % fry hatched is defined as

$$\frac{\text{no. of fry hatched by that day}}{\text{no. of fry ultimately hatched}} \times 100$$

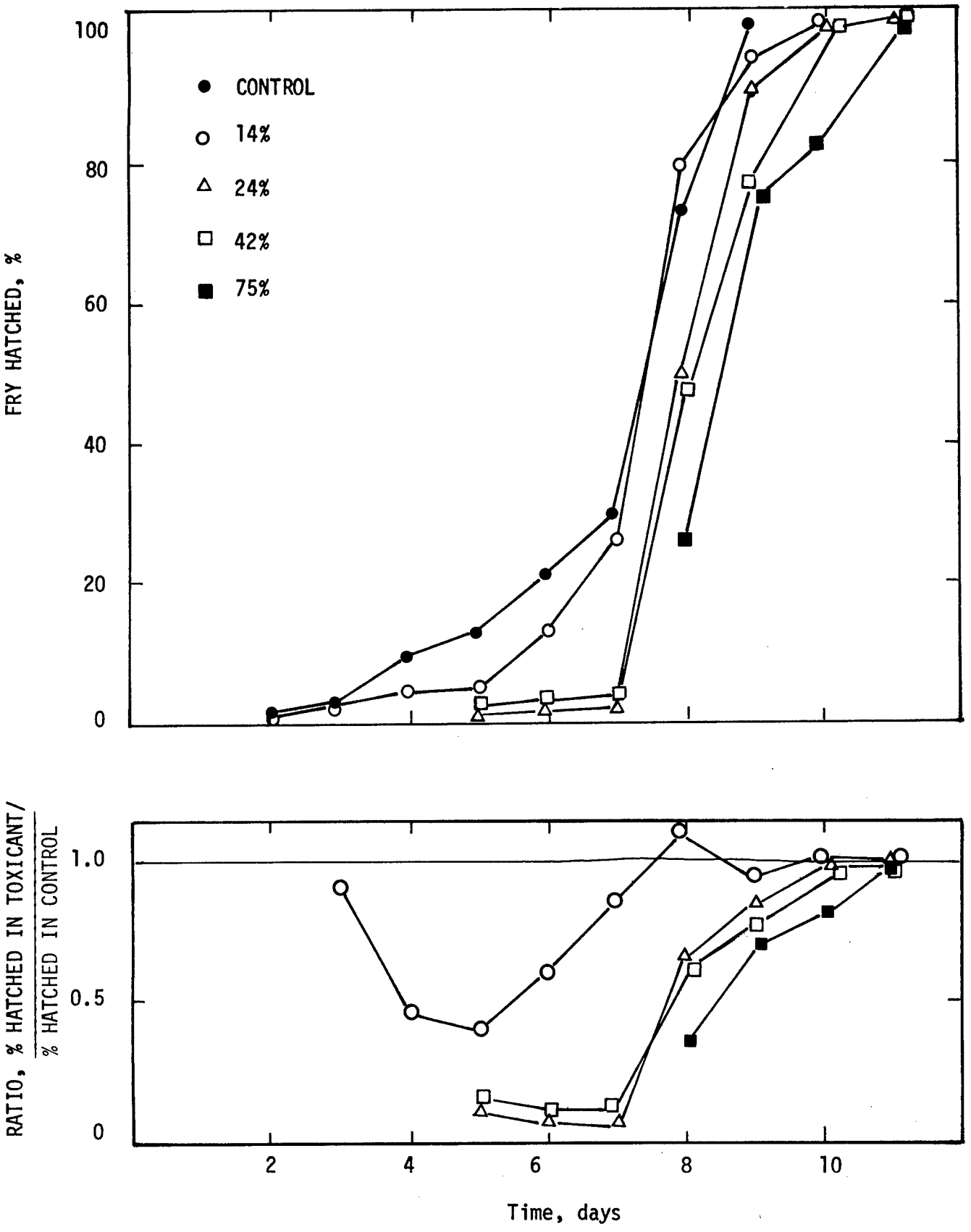



FIGURE 10. PERCENT HATCHABILITY TO FLAGFISH FRY IN WSF OF JP-8 AND RATIO OF PERCENT HATCHABILITY IN TOXICANT TO PERCENT HATCHABILITY IN CONTROL

There is 100% hatchability in all tanks by day 11, but evidence that the presence of toxicant (WSF of JP-8) retards hatchability is demonstrated in the bottom graph. This depicts the ratio of % hatchability in the various % toxicants divided by the % hatchability in the control plotted vs. time. The results indicate retardation in the 24 and 42% tanks for 9 days and more severe retardation in the 75% tank for 10 days. Despite the evidence of retardation, about three-fourths of the eggs in all of the tanks hatched between days 7 and 9. The ultimate effect was to delay 100% hatchability by 24 hr in the 14, 24, and 42% toxicant tanks and by 48 hr in the 75% toxicant tank.

The daily exchange of egg cups enabled the eggs in the 75% toxicant to hatch. However, these hatched fry appeared curled up and were small and inactive compared to the fry from other concentrations. The extreme cleaning measures needed to allow them to hatch indicates the weakness of these eggs and their poor resistance to disease.

The survivability of the newly hatched fry on day 14 is presented in Table 6. It is apparent that 75% toxicant prohibits the successful hatching of eggs to viable fry. Eggs hatching in this toxicant concentration produce deformed fry which quickly die (within 3 days after hatching). These eggs were extremely susceptible to fungal infection, and no such effects were seen in the more dilute toxicant concentrations.

Table 6

Viable Fry on Day 14

| Tank | WSF of JP-8,% | Viability of Hatched Fry, % |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 14 | 94 |
| 3 | 24 | 98 |
| 4 | 42 | 95 |
| 5 | 75 | 0 |

Fry lengths were measured photographically on days 10 and 24 (Table 7). On day 10 the fry exposed to 42% toxicant were significantly (at the 99% level) smaller than the control. By day 24 the fry in the 24% toxicant were also significantly smaller than in the control. There was no significant difference in size between the fry exposed to 14% toxicant and the control on day 10. However, for unknown reasons a significant die-off occurred at this concentration on day 24 so that size comparisons were not possible. Shortly thereafter a mass mortality occurred in all tanks except the control again for the unknown reasons.

Measured JP-8 concentrations in the tanks are indicated in Table 6 for the period of the hatchability study.

Table 7

Length of Fry on Days 10 and 24

| WSF of JP-8, Concentration % | JP-8, mg/ℓ ± s (n=6) | Fry Measurements | | | |
|---|---|---|---|---|---|
| | | Day 10 | | Day 24 | |
| | | No. | Length (mm) ± s | No. | Length (mm) ± s |
| 0 | 0 | 72 | 3.8 ± 0.4 | 89 | 5.5 ± 0.7 |
| 14 | 1.0 ± 0.2 | 92 | 3.8 ± 0.3 | 18 | 5.1 ± 0.2 |
| 24 | 1.7 ± 0.7 | 102 | 3.7 ± 0.3 | 108 | 5.0 ± 0.4 |
| 42 | 3.1 ± 1.1 | 88 | 3.3 ± 0.4 | 62 | 4.9 ± 0.5 |
| 75 | 6.8 ± 1.1 | 0 | -- | 0 | -- |

DISCUSSION OF HATCHABILITY RESULTS

The following conclusions may be drawn concerning the effect of JP-8 on flagfish egg hatchability.

1. No viable fry hatched at 6.8 ± 1.1 mg/ℓ JP-8.

2. There was no interference in the ultimate percentage hatchability to viable fry at WSF of JP-8 concentrations in the range of 0-3.1 mg/ℓ, although there was evidence of significant retardation in hatchability at JP-8 concentrations of 1.7 mg/ℓ and above.

3. At 1.0 + 0.2 mg/ℓ WSF of JP-8 there was no effect on flagfish egg hatchability other than minor retardation during the first 6 days affecting less than 7% of the eggs ultimately hatched.

4. A significant difference in fry size between the control fry and fry hatched in a concentration of 3.1 mg/ℓ JP-8 existed as early as day 10. By day 24 the fry hatched in a concentration of 1.7 mg/ℓ JP-8 were also significantly smaller than the control fry.

RESULTS OF GROWTH AND DEVELOPMENT STUDY

From two of the early hatchability studies there were sufficient fry to initiate a growth and development study. A staggered start was used for each of the two banks of 5 tanks designated as Bank A and Bank B. There was a 6-day age difference between the fry in the two banks of tanks which contained

duplicate target ranges of WSF of fuel concentrations of 14, 24, 42, and 75% generated by adding dilutions of 16, 29, 48 and 86%.

Figure 11 is a flow diagram indicating numbers of fry introduced initially, their survival during each phase of the study, and the time of transfer from egg cup to fry chamber to exposure tank. Specifically, flagfish eggs were hatched in egg cups in the bank B tanks and viable fry that resulted were transferred to fry chambers on day 15 after placement of eggs. At this time there were 21 fry in the chamber of tank 1 (0%), 27 fry in the chamber of tank 2 (14%), 27 fry in the chamber of tank 3 (24%), 25 fry in the chamber of tank 4 (42%), and 15 fry in the chamber of tank 5 (75%). To supply fry for tank 5, 15 fry were transferred from tank 4, since no viable fry had been hatched in tank 5.

Bank A tanks received flagfish eggs 6 days after bank B and on day 15 after placement of eggs the following number of fry were transferred from egg cups to fry chambers: 40 fry to chamber 1 (0%), 14 fry to chamber 2 (14%), 39 fry to chamber 3 (24%), 30 fry to chamber 4 (42%) and 26 fry to chamber 5 (75%). Again, it was necessary to transfer fry into chamber 5 to study growth and development because no viable fry could be hatched at that concentration.

During the first 3 weeks of the study, including the hatchability period, 4 sets of GC measurements were taken to assess the actual JP-8 concentrations in the tanks. The results (Table 8) show reasonably close duplication of concentrations between the two banks of tanks. The measured % of the WSF was computed based on the GC measurement of JP-8 in each tank divided by the measured JP-8 concentration of 100% WSF from the solubilizer. The values obtained are close to the desired percentages. Throughout the 4-month duration of the bioassay, the flow rates of JP-8 WSF and dilution water were measured and adjusted daily to maintain the desired % WSF in each tank.

The fry resided in the fry chambers until day 59 and were then transferred out of the chambers into the tanks to avoid overcrowding. While they were in the fry chambers photographic measurements were taken periodically to assess fry growth by length measurements. After the fry were allowed to swim freely in the open tanks it was not feasible to remove them from the tanks so that no further length measurements could be made until the termination of the study on day 128.

Both banks of tanks were photographed on day 15, i.e. 6 days apart - because of the staggered start, but thereafter photographs were taken on the same day for convenience. Thus, Bank A was photographed on days 25, 38, and 53 and Bank B was photographed on days 31, 44 and 59.

Results of fry size measurements taken during the fry chamber period and at the conclusion of the study are presented in Table 9 for Bank A and Table 10 for Bank B. The JP-8 concentrations given for each tank represent the mean of 15 measurements for Bank and and 17 measurements for Bank B taken at weekly intervals throughout the 128-day bioassay. No size measurements were taken of the fry transferred into tank 5A and 5B because the fry did not survive.

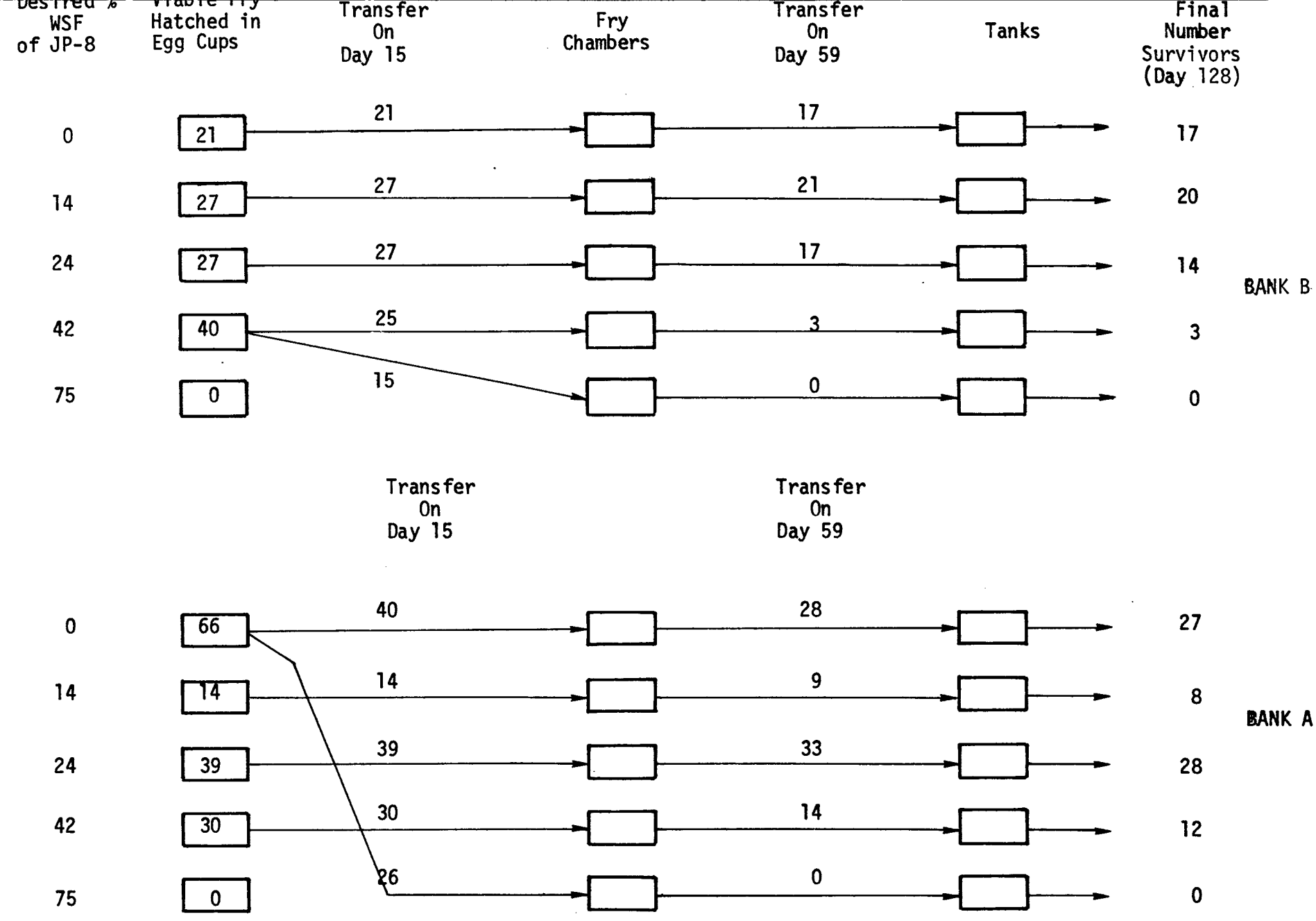



FIGURE 11. FLOW DIAGRAM OF CHRONIC FLAGFISH BIOASSAY — WSF OF JP-8

Table 8

JP-8 Concentrations During the Initial Phase of the Growth and Development Study

| Sample | Measured JP-8, mg/ℓ ± s | % of WSF | |
|---|---|---|---|
| | | Desired | Measured ± s |
| 100% WSF | 10.64 ± 1.57 | | |
| Tank 1A | 0 | 0 | 0 |
| Tank 2A | 1.20 ± 0.42 | 14 | 11 ± 3.9 |
| Tank 3A | 2.71 ± 1.23 | 24 | 25 ± 11.6 |
| Tank 4A | 4.74 ± 0.82 | 42 | 45 ± 7.7 |
| Tank 5A | 8.23 ± 1.24 | 75 | 77 ± 11.7 |
| Tank 1B | 0 | 0 | 0 |
| Tank 2B | 1.13 ± 0.43 | 14 | 11 ± 4.0 |
| Tank 3B | 2.37 ± 1.00 | 24 | 22 ± 9.4 |
| Tank 4B | 4.21 ± 0.79 | 42 | 40 ± 7.4 |
| Tank 5B | 8.27 ± 0.32 | 75 | 78 ± 3.0 |

There is clear evidence of retarded fry growth at JP-8 WSF concentrations of approximately 1.5 mg/ℓ (Tanks 3A and 3B) by the end of the fry chamber period. At higher JP-8 concentrations (approximately 3 mg/ℓ in Tanks 4A and 4B) there are indications of retarded growth as early as day 25.

The significance of the differences in fry size was evaluated at the 99% confidence level by the student t-test. On day 38 and thereafter there was a significant difference at 3 mg/ℓ JP-8. By the end of the fry chamber period the t-test indicated significantly smaller fry at ~ 1.5 mg/ℓ JP-8 (tanks 3A and 3B) compared to the controls, but results for tank 2 (~1 mg/ℓ JP-8) are not well defined. Tank 2A fry were not significantly different in size from the control fry which may be attributable to less competition for food among the 9 fry in Tank 2A than among the 28 fry in the 1A control. The reverse is true when the fry in Tank 2B are compared with the fry in the 1B control. The size difference is statistically significant at the 99% level, but there are more fry (21) in 2B than in the 1B control (15).

Fry growth rate is depicted in Figure 12 for tanks 1, 2, 3 and 4 of each bank. The mean sizes of fry in Bank A tanks are plotted on days 25, 38, 53 and 128, and the mean sizes of fry in Bank B tanks are plotted on days 31, 44, 59 and 128. The two banks appear to duplicate each other reasonably well with respect to fry growth rate.

The final length measurements taken at the conclusion of the study do not indicate as great a size difference between means as noted during the fry chamber period. The difference in means was significant at the 97.5% confidence level between the control and 42% tanks, but there was not a significant difference at 95% between the means of the control and the 14 or 24% tanks.

Thus, the retardation in growth appeared to be overcome as time progressed. Survival of flagfish during the test was assessed by combining Tank A and B data for each JP-8 concentration. There was 72% survival in the controls, 68% survival at 14% JP-8, 64% survival at 24% JP-8, 27% survival at 42% JP-8 and no survival at 75% JP-8. There was no significant difference in the survivals at or below the 24% JP-8 concentration equivalent to 1.5 mg/ℓ JP-8.

Results of mean measured JP-8 concentrations (Tables 9 and 10) may be used to compute the actual percent WSF of JP-8 relative to the mean solubilizer output of 10.9 mg/ℓ WSF of JP-8. The computed percentages for each tank are presented in Table 11. The actual result in each case is lower than the desired value, and this loss cannot be attributed to the metering of dilution water and JP-8 WSF which was measured daily and was seldom in need of minor adjustment. Neither can the loss be attributable to volatility which was compensated for in the manner previously explained. Rather, it seems likely that the susceptibility of JP-8 to biodegradation may account for the depletion of fuel in the tanks.

The ease with which JP-8 is biodegraded was demonstrated in a separate BOD experiment. Between the weekly cleanings administered to the JP-8 solubilizer a flocculent-type of material buildup and clings to interior surface of the glass tubing. Using a measured quantity of this material added to the

Table 9

Fry Growth in Bank A Tanks of Chronic Flagfish Bioassay

| Tank | JP-8 mg/ℓ (n=15) ± s | Fry Growth | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 15 | | Day 31 | | Day 44 | | Day 59 | | Day 128 | |
| | | No. | Length, mm ± s | No. | Length, mm ± s | No. | Length, mm ± s | No. | Length mm ± s | No. | Length, mm ± s |
| 1 | 0 | 40 | 3.5 ±0.3 | 31 | 5.4 ±0.5 | 29 | 9.0 ±0.5 | 28 | 13.3 ± 1.9 | 27 | 26.6 ± 2.9 |
| 2 | 0.97 ±0.20 | 14 | 3.5 ±0.4 | 9 | 5.1 ±0.2 | 9 | 9.0 ±1.0 | 9 | 13.5 ±1.8 | 8 | 29.2 ± 1.8 |
| 3 | 1.46 ±0.50 | 39 | 3.8 ±0.2 | 33 | 5.6 ±0.5 | 33 | 8.7 ±1.5 | 33 | 11.6 ± 1.5 | 28 | 25.5 ± 2.8 |
| 4 | 3.02 ±0.96 | 30 | 3.7 ±0.3 | 15 | 4.8 ±0.4 | 14 | 7.3 ±0.7 | 14 | 11.1 ± 1.3 | 12 | 24.2 ± 2.5 |
| 5 | 6.48 ±1.18 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |

Table 10

Fry Growth in Bank B Tanks of Chronic Flagfish Bioassay

| Tank | JP-8 mg/ℓ (n=17) ± s | Fry Growth | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 15 | | Day 25 | | Day 38 | | Day 53 | | Day 128 | |
| | | No. | Length, mm ± s | No. | Length, mm ± s | No. | Length, mm ± s | No. | Length, mm ± s | No. | Length, mm ± s |
| 1 | 0 | 21 | 3.8 ±0.3 | 19 | 6.4 ±0.4 | 18 | 10.6 ± 0.7 | 17 | 14.5 ± 1.5 | 17 | 28.2 ± 2.6 |
| 2 | 1.00 ±0.33 | 27 | 3.8 ±0.3 | 21 | 6.2 ±0.3 | 21 | 9.2 ±1.0 | 21 | 13.2 ± 1.1 | 20 | 25.9 ± 2.5 |
| 3 | 1.58 ±0.72 | 27 | 3.8 ±0.5 | 17 | 5.9 ±0.5 | 17 | 8.7 ±0.9 | 17 | 13.0 ± 1.6 | 14 | 26.7 ± 2.7 |
| 4 | 3.33 ±1.04 | 25 | 3.6 ±0.4 | 3 | 5.3 ±0.9 | 3 | 7.3 ±0.6 | 3 | 12.3 ± 0.4 | 3 | 24.6 ± 1.5 |
| 5 | 7.19 ±1.72 | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |

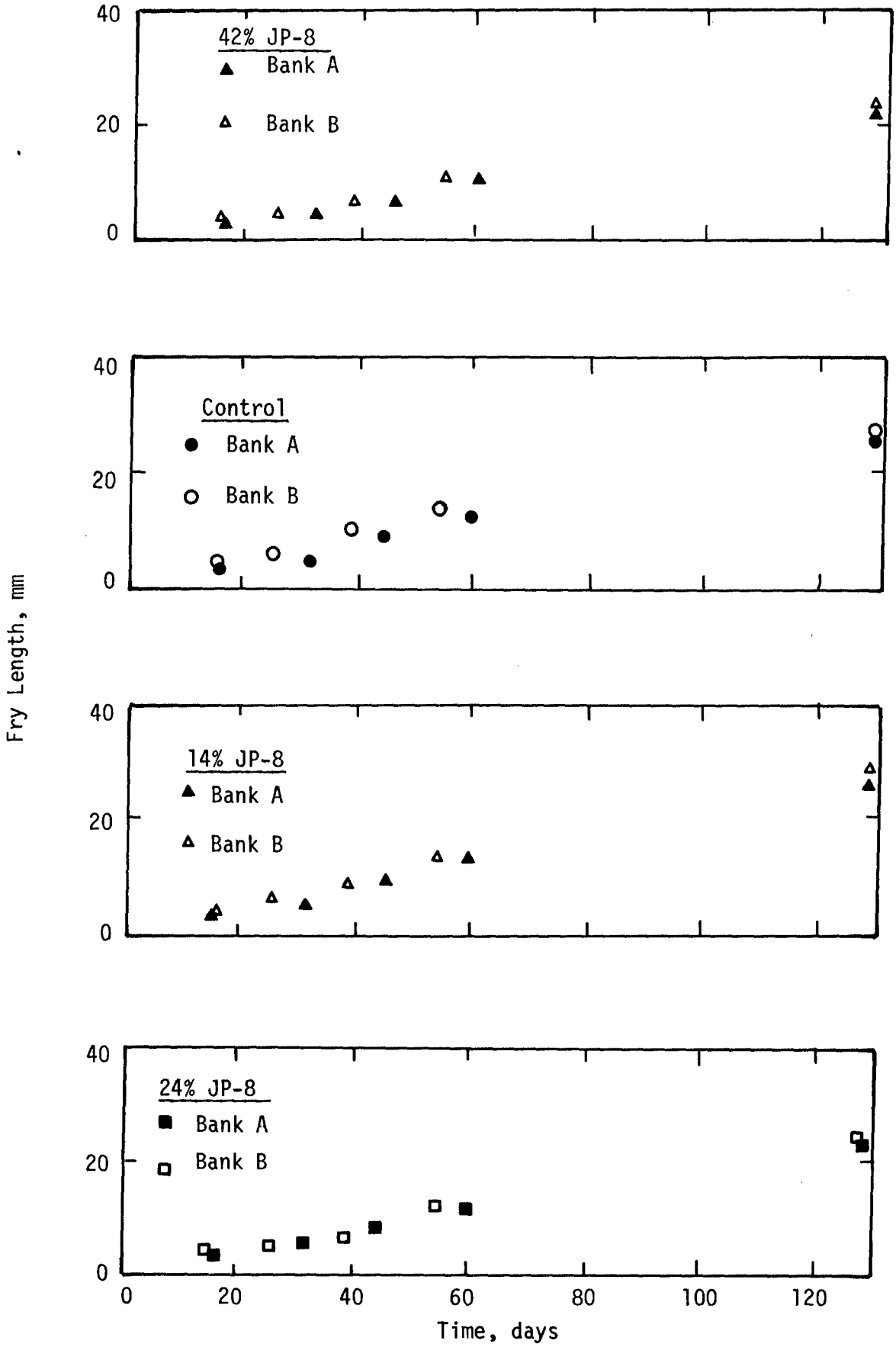



FIGURE 12. DUPLICATION OF FRY GROWTH RATE BETWEEN BANK A AND BANK B TANKS.

WSF of JP-8, the BOD was determined before and after the system was cleaned. The "before cleaning" sample had BOD's of 2, 3 and 4 mg/ℓ after 2, 4 and 8 days, respectively. The "after cleaning" sample had BOD's of 0 and 2 mg/ℓ after 3 and 7 days, respectively. The conclusion is that this growth contains a bacterial culture which is adapted to JP-8.

Table 11

Actual vs. Nominal % WSF of JP-8

| Nominal of WSF of JP-8 | Actual % WSF of JP-8 ± s | |
|---|---|---|
| | Bank A | Bank B |
| 0 | 0 | 0 |
| 14 | 8.9 ± 1.8 | 9.2 ± 3.0 |
| 24 | 13.4 ± 4.6 | 14.5 ± 6.6 |
| 42 | 27.7 ± 8.8 | 30.6 ± 9.5 |
| 75 | 59.4 ±10.8 | 66.0 ±15.8 |

RESULTS OF FUEL ACCUMULATION

Accumulation of JP-8 in the whole body of flagfish exposed to the 128-day chronic bioassay was examined. Because these fish were small (mean wet wt. = 0.36g) the examination of specific tissues was not possible. For this reason a study was conducted in which larger (1-4g wet wt.) flagfish were taken from the breeding room and exposed to 42% WSF of JP-8 for 14 days. They were examined periodically for JP-8 accumulation in the muscle, liver and whole body.

Results of Chronic Bioassay JP-8 Accumulation

Upon termination of the chronic bioassay, flagfish were removed from the exposure tanks and analyzed for JP-8. All fish from a given tank were combined to provide a sample of sufficient size to permit reliable quantification. Figure 13 is a plot of the JP-8 concentration in fish whole body tissue as a function of the mean JP-8 concentration in the exposure tank. The correlation coefficient, r, is 0.803 indicating at the 95% confidence level that a correlation exists between JP-8 levels in fish whole body tissue and JP-8 aqueous concentration. The equation of the linear regression is Y = 138.7X + 27.5.

Table 12 shows that the ratio of JP-8 in fish tissue to JP-8 concentration in the aqueous environments varies between 133-220 (mean = 159).

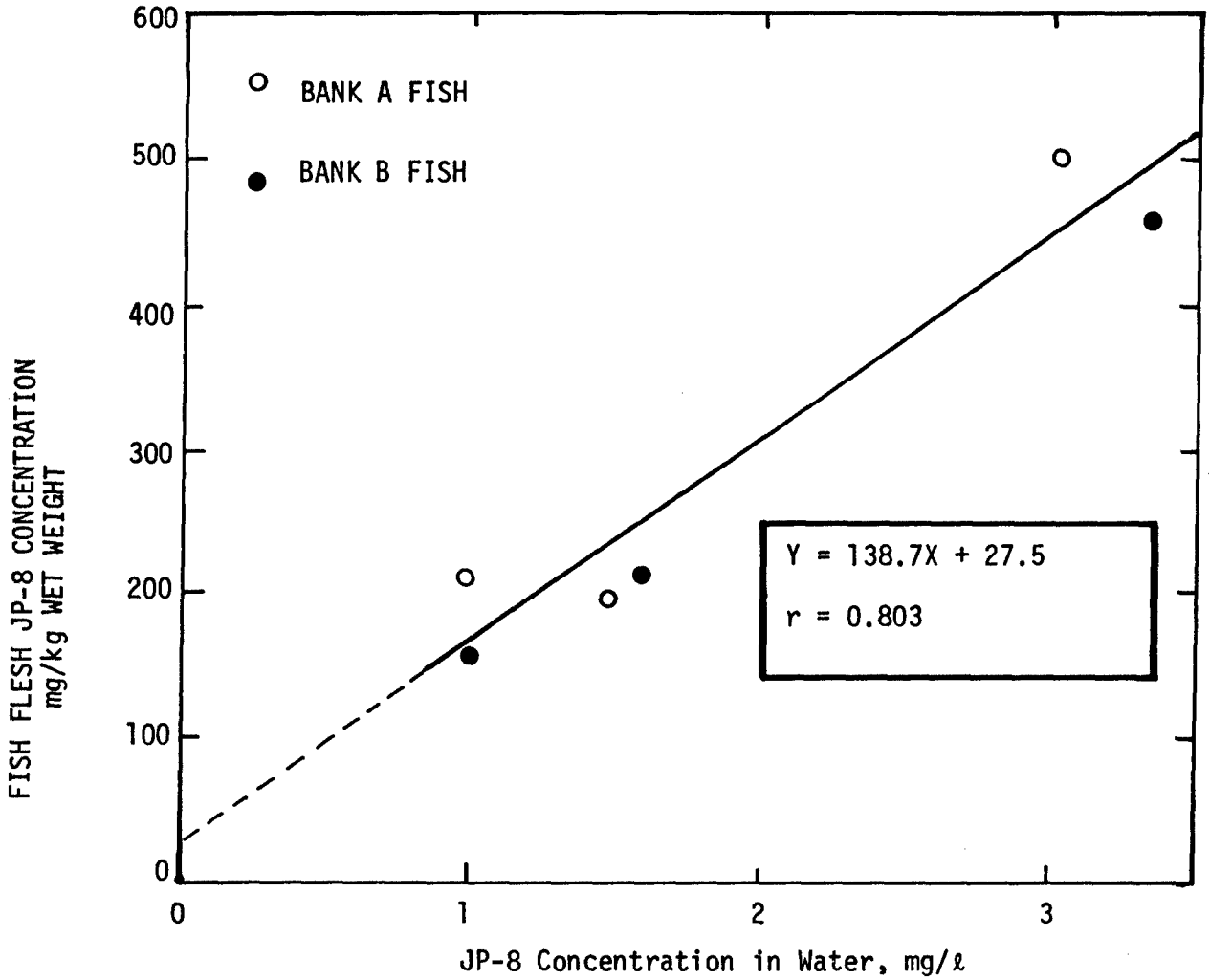



FIGURE 13. ACCUMULATION OF JP-8 IN FLAGFISH WHOLE BODY TISSUE AFTER 128 DAYS EXPOSURE TO WSF OF JP-8

Table 12

Ratio of JP-8 Concentrations in Flagfish
Whole Body Tissue to Water JP-8 Concentration (128 Days' Exposure)

| EXPOSURE TANK | BANK A | | | BANK B | | |
|---|---|---|---|---|---|---|
| | WSF of JP-8 mg/ℓ ± s | JP-8 in Tissue mg/Kg wet wt. | Ratio mg/Kg / mg/ℓ | WSF of JP-8 mg/ℓ ± s | JP-8 in Tissue mg/Kg wet wt. | Ratio mg/Kg / mg/ℓ |
| 2 | 0.97 ±0.20 | 213 | 220 | 1.00 ±0.33 | 161 | 161 |
| 3 | 1.46 ±0.50 | 197 | 135 | 1.58 ±0.72 | 210 | 133 |
| 4 | 3.02 ±0.96 | 501 | 166 | 3.33 ±1.04 | 458 | 138 |

Table 13

JP-8 Concentration in Flagfish Whole Body, Muscle and Liver Tissue

| Time, days | JP-8 Concentration, mg/Kg wet wt. | | |
|---|---|---|---|
| | Whole Body | Muscle | Liver |
| 1 | 33 | 21 | |
| 3 | 203 | | |
| 7 | 185 | | |
| 14 | 329 | 165 | 448 |
| | Purge (Fuel-free water) | | |
| 3 | 185 | | |
| 7 | 116 | | |
| 14 | 38 | | |

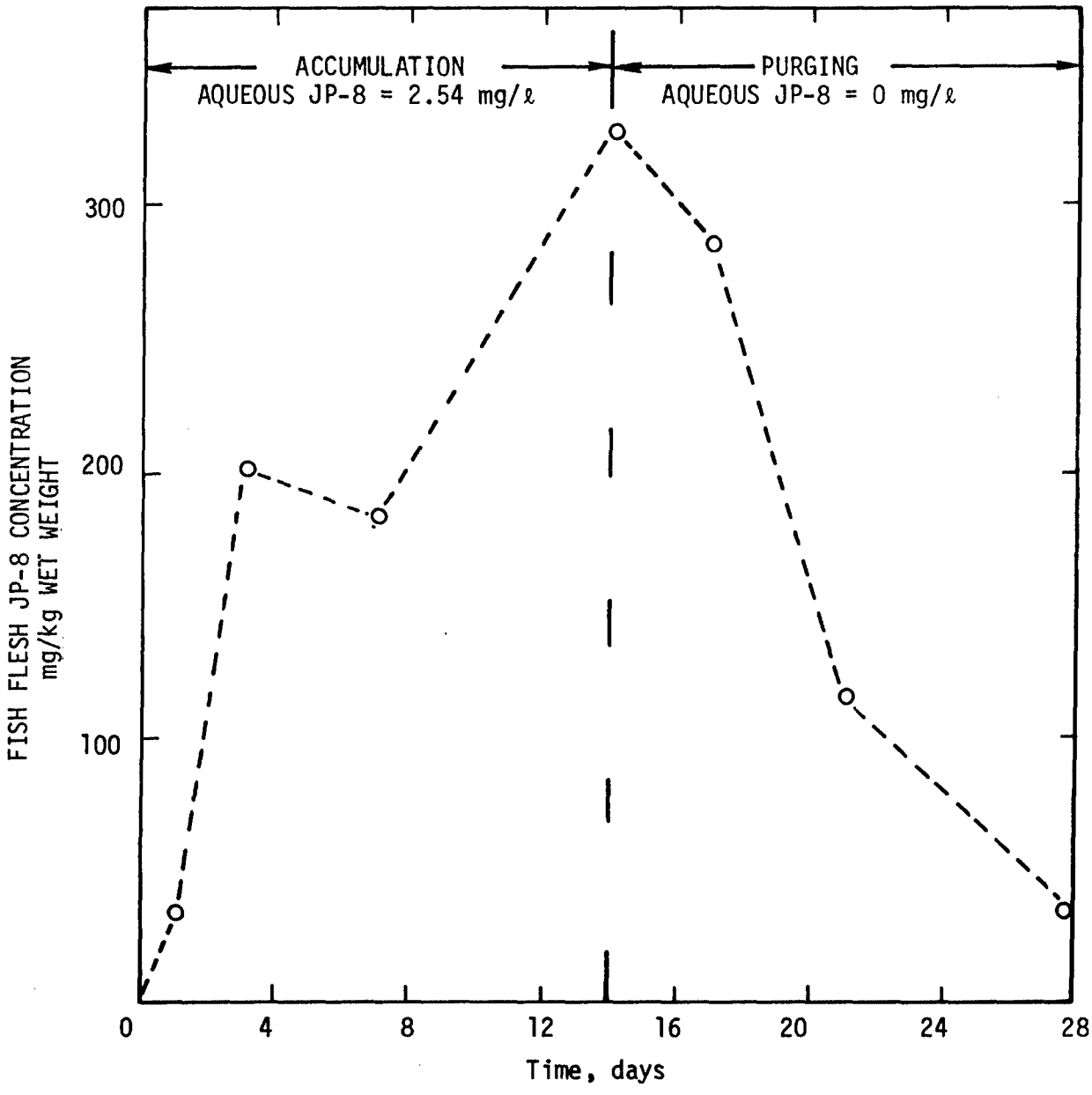



FIGURE 14. ACCUMULATION AND PURGING RATE OF JP-8 IN FLAGFISH WHOLE BODY TISSUE

JP-8 Accumulation and Purging

A total of 70 adult flagfish were placed in a 42% WSF of JP-8 exposure tank (JP-8 = 2.54 mg/ℓ). JP-8 accumulation was examined in the liver after 14 days, in the muscle tissue after 1 and 14 days, and in the whole body tissue after 1, 3, 7 and 14 days. The remaining fish were placed in fuel-free water and the whole body tissue was examined after 3, 7 and 14 days of "purging".

There was little accumulation in whole body and muscle tissue after 1 day's exposure but substantial accumulation after 3 days exposure (approximately 200 mg per kg of wet whole body tissue) (Table 13).

By day 14 the accumulation ratio in whole body tissue was 129.5 ___ a value that approaches those determined in the chronic bioassay after 128 days exposure. After 14 days exposure, JP-8 appears to be preferentially accumulated in liver tissue (448 mg per kg wet wt.) compared to muscle tissue (165 mg per kg wet wt.).

The rate of accumulation and purging of JP-8 was determined for whole body tissue only, since there were insufficient fish to examine the rate of purging from liver or muscle tissue. The JP-8 levels in whole body tissue had after 3 days purging decreased by about 10%, by about 65% JP-8 after 7 days, and by about 90% after 14 days, (Figure 14).

DISCUSSION

During the growth and development phase of the continuous-flow chronic bioassay, toxic effects appeared at WSF of JP-8 concentrations of 3 mg/ℓ and greater. Toxic effects at 3 mg/ℓ were both gross and subtle, including a significantly higher mortality rate and a significantly smaller length after 10 days than flagfish exposed to JP-8 concentrations of less than 3 mg/ℓ.

At a JP-8 concentration of approximately 1.5 mg/ℓ there was some evidence of retarded growth during two months of exposure. After three and four months of exposure however, fish in 1.5 mg/ℓ JP-8 show no significant difference in length from the controls. Flagfish exposed to 1 mg/ℓ JP-8 grew similarly to the controls for the entire experiment. Based on these results the no effect level with respect to growth is between 1.5 and 3.0 mg/ℓ JP-8.

The only abnormal development observed was the deformed fry produced during the hatchability period in the presence of 6.8 ± 1.1 mg/ℓ JP-8.

No assessment can be made of mutagenic effects because the flagfish would not reproduce in the continuous-flow exposure tanks.

JP-8 accumulates in the whole body tissue of flagfish. Equilibrium concentrations are reached after approximately two weeks of exposure. There is a linear relationship between whole body JP-8 concentration and solution concentration of JP-8. The JP-8 is concentrated about 160 times (158.8 ± 33.1) in whole body tissue. The concentration of JP-8 in liver tissue is approximately 2.5 times greater than in muscle tissue. JP-8 is not purged

rapidly from whole body tissue when fish with accumulated JP-8 are permitted to swim in fuel-free water. JP-8 is depleted from whole body tissue at the rate of about 7% per day.

## 6. HYDRAZINE STUDIES

### RANGE-FINDING STUDY

A preliminary range-finding study was conducted to determine the approximate concentration at which hydrazine will cause stickleback mortality. This estimate was used to establish the concentration range for spill tests in the analog tanks and for acute static bioassays in 5-gal jars.

A series of hydrazine concentrations of 0, 5, 15, and 30 mg/ℓ was studied for a 260-hr period using 1-gal capacity jars, each containing three sticklebacks. The jars were covered with aluminum foil and aerated gently during the course of the assay.

Results (Table 14) indicated that the 96-hr LC 50 may be on the order of 5 mg/ℓ. Consequently future studies will be conducted in the 0-10 mg/ℓ range. The survival of the fish in the 5 mg/ℓ jar may be due to rapid degradation of hydrazine as indicated by the decay study previously reported. In future studies the hydrazine concentration will be renewed daily to avoid the decay problem.

Table 14

Acute Toxicity of Hydrazine to Sticklebacks (Range-Finding Study)

| Hydrazine Concentration mg/ℓ | Fish Survival, Number | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time in hours | | | | | | |
| | 0 | 1 | 18 | 21 | 25 | 44 | 260 |
| 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 5 | 3 | 3 | 2 | 2 | 2 | 2 | 2 |
| 15 | 3 | 2 | 0 | 0 | 0 | 0 | 0 |
| 30 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |

### JAR-TEST

A static acute bioassay was conducted to determine the 96-hr LC 50 of sticklebacks to hydrazine.

Nine to ten sticklebacks (Gasterosteus aculeatus) were added to each of 12 5-gal jugs containing 16ℓ of Bay water. Six duplicate hydrazine concentrations in the range of 0 to 10 mg/ℓ were investigated.

The jars, covered with aluminum foil, were gently aerated from house air supply and maintained at approximately 15 C. The hydrazine solutions were renewed daily, and routine measurements were taken of DO, pH and temperature; pH was in the range 7.65 to 8.00, DO in the range 6.5 to 7.8 and temperature in the range of 14.0 to 15.5 C. The salinity of the Bay water was 18 g/ℓ.

Stickleback survival, Table 15, followed the expected progression of increased mortality with increasing toxicant levels, and the replication was satisfactory. The LC 50 at 24-hr was 8.5 mg/ℓ, at 48-hr was 7.5 and 5.0 mg/ℓ, and at 96-hr was 3.6 and 3.2 mg/ℓ. The mean 96-hr LC 50 of the duplicates was 3.4 mg/ℓ (nominal concentrations).

The loss of hydrazine after the initial 24-hr period was measured to ascertain whether the degradation rate was consistent with previous determinations. The loss shown in Table 15, indicated that in the critical range of 3.2 mg/ℓ the hydrazine degraded to approximately 2.2 mg/ℓ in 24 hr.

The test was not repeated for each 24 hr period after each renewal, but it is likely that this order of degradation remained consistent. Therefore, the actual 96-hr LC 50 was undoubtedly less than the 3.4 mg/ℓ specified. Because of significant degradation the need for continuous flow bioassays for hydrazine is evident.

## ANALOG FISH AND AUFWUCHS STATIC BIOASSAY

### Introduction

The effects of a hydrazine spill were simulated by adding a single dose of hydrazine to an analog tank operated as a static (non-flow through) unit.

### Procedure

A rack containing 30 aufwuchs substrates was suspended 50 cm beneath the water surface of each of seven tanks. The tanks were operated on a continuous flow of Bay water at a rate of 4.8ℓ/min for two weeks to establish aufwuchs growth.

Table 15
Acute Toxicity of Hydrazine to Sticklebacks - Jar Test

| Hydrazine Concentration, mg/ℓ | | | Fish Survival, No. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | After 24 hr | | Time, hr | | | | | | | | | |
| Desired | Measured | | 0 | | 24 | | 48 | | 72 | | 96 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| 0 | 0 | 0 | 9 | 10 | 9 | 10 | 9 | 10 | 9 | 10 | 9 | 10 |
| 1 | 0.36 | 0.47 | 9 | 10 | 9 | 10 | 9 | 10 | 9 | 10 | 9 | 10 |
| 1.8 | 0.77 | 0.95 | 9 | 10 | 9 | 10 | 9 | 10 | 9 | 10 | 9 | 10 |
| 3.2 | 2.19 | 2.13 | 9 | 10 | 9 | 10 | 9 | 10 | 9 | 10 | 6 | 5 |
| 5.6 | 4.49 | 4.66 | 9 | 10 | 9 | 10 | 8 | 9 | 1 | 1 | 0 | 0 |
| 10.0 | 8.55 | 8.04 | 9 | 10 | 6 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |

The nominal hydraulic residence time at this flow rate was 14.58 hr. Bay water flow was then terminated and 8 of the aufwuchs growth units were removed from each tank and examined for metabolic response, chlorophyll a content and biomass. At this time also fish cages each containing 10 fish (stickleback) were placed in each analog tank. Two tanks were dosed with hydrazine to produce nominal hydrazine concentrations of 10 mg/ℓ; two tanks were dosed to produce hydrazine concentrations of 5.6 mg/ℓ; one tank was dosed to produce a hydrazine concentration of 3.2 mg/ℓ; two control tanks received no hydrazine. Hydrazine concentrations were measured 2 hrs after hydrazine introduction and periodically thereafter for the 240 hr duration of the experiment. Air was slowly bubbled into the tanks throughout the assay period, and DO measurements were taken daily. The fish were observed daily for mortality and signs of stress. Eight aufwuchs growth units were removed from each tank 48 hr and 96 hrs after hydrazine introduction and on each occasion analyzed for metabolic response, chlorophyll a content and biomass.

## Results

Hydrazine concentrations measured throughout the study are presented in Figure 15. The measured values after 2 hr were close to the desired initial concentrations. Analog tank "F", although starting with a nominal hydrazine concentration of 10 mg/ℓ rapidly fell to lower levels because of leakage through a partition between a neighboring vigorously aerated fish rearing tank. The vigorous aeration increased hydrazine loss by oxidation and volatilization causing a more rapid decrease in hydrazine concentration than would be anticipated from dilution alone. All of the other tanks exhibited a decline in hydrazine concentration at a rate consistent with the preliminary range-finding and jar test studies.

Significant stickleback deaths occurred in all hydrazine-dosed tanks with the exception of tank F (Table 16). During a 96-hr exposure period significant mortality occurred only in the tanks containing the two highest hydrazine concentrations (5.6 and 10 mg/ℓ). Fish deaths continued to occur in all hydrazine levels tested. In the lowest initial concentration tested (3.2 mg/ℓ) there was a 60% mortality after 15 days exposure. In tank F where hydrazine was lost rapidly the sticklebacks were able to survive (with one exception) for the duration of the experiment. This indicates that the fish could survive a high initial dose of hydrazine if it were quickly removed. On days 2 and 3 some of the fish in tank F showed signs of stress such as difficulty in maintaining equilibrium, but by day 4 they had recovered. Sticklebacks in Tank D (initial hydrazine concentration of 3.2 mg/ℓ) were dying when the hydrazine concentration in the tank was very low (approximately 0.5 to 1 mg/ℓ) indicating that a prolonged exposure to this hydrazine concentration may be more toxic than a very short exposure to a higher (10 mg/ℓ) hydrazine level.

The 96-hr LC 50, based on the initial desired concentrations, was 6.6 mg/ℓ hydrazine with 95% confidence limits of 5.0 to 8.8 mg/ℓ hydrazine. The results in analog "F" were ignored in this computation. The 96-hr LC 50 is essentially useless because of the demonstrated degradation of hydrazine.

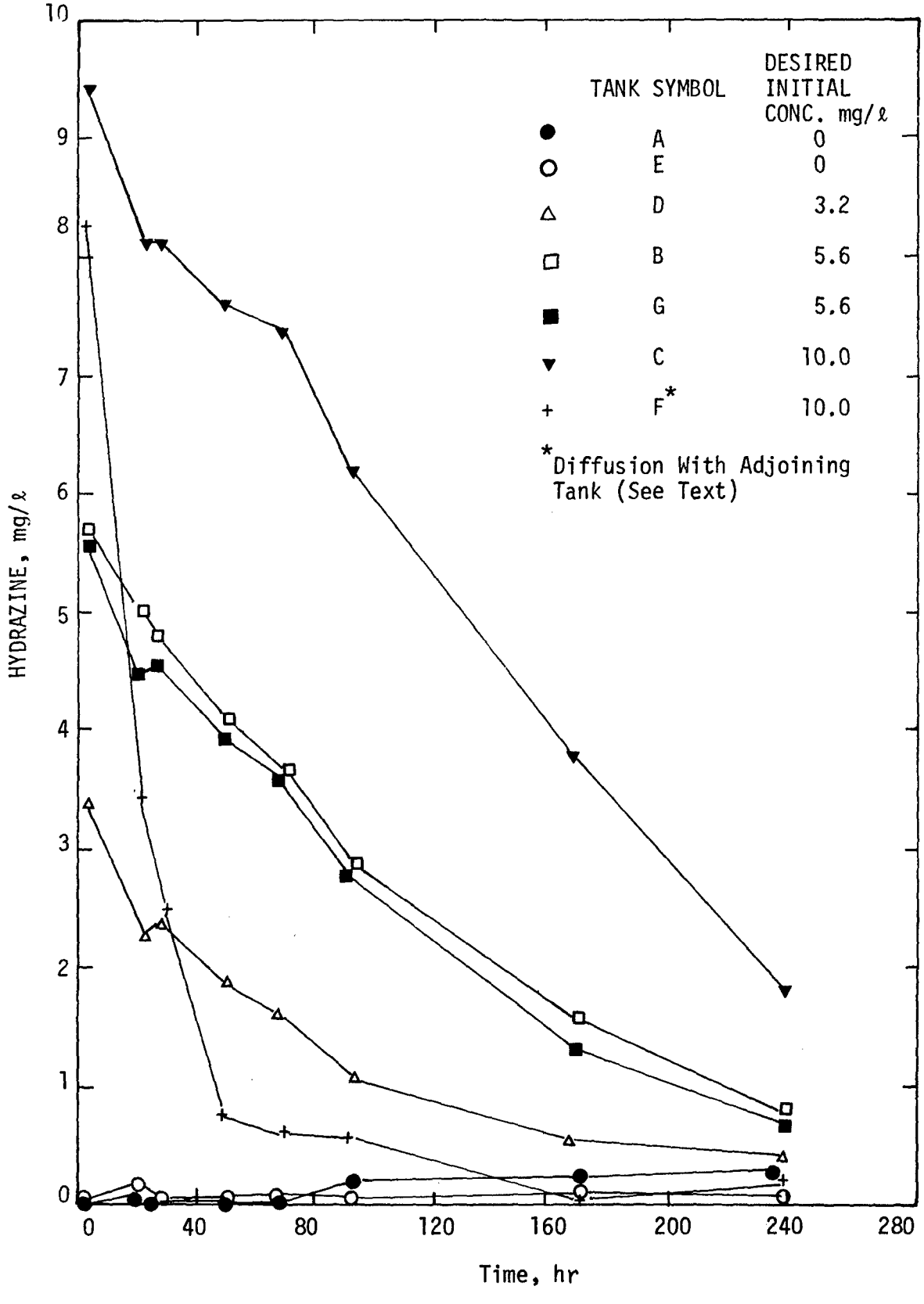



FIGURE 15. HYDRAZINE CONCENTRATION IN "SPILL" BIOASSAY USING STICKLEBACKS AND AUFWUCHS

Table 16

Survival of Sticklebacks in Analog Tank Hydrazine Bioassay

| Analog Symbol | Desired Initial Hydrazine Concentration mg/ℓ | Number of Fish Surviving | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 9 | 10 | 12 | 15 |
| A | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| E | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| D | 3.2 | 10 | 10 | 10 | 10 | 10 | 9 | 8 | 5 | 4 |
| B | 5.6 | 10 | 10 | 9 | 6 | 6 | 3 | 3 | 0 | 0 |
| G | 5.6 | 10 | 10 | 8 | 8 | 6 | 2 | 2 | 2 | 2 |
| C | 10 | 10 | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 10* | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 9 |

*See text-leaky partition-diffusion with adjoining tank (fish rearing tank)

The biomass of the standing crop of aufwuchs (on the three occasions examined) is reported in Table 17 and the variability of the crop among tanks is indicated. Table 18 indicates all concentrations of hydrazine tested appear to severely affect aufwuchs. The values 48 hrs after hydrazine addition indicate that most aufwuchs exposed to hydrazine are no longer viable. The P.I's at all hydrazine concentrations are markedly reduced from the initial examination, while the controls are reduced to a lesser extent. There is also some evidence that the amount of chlorophyll a per dry weight is reduced.

The analysis after 96 hrs shows the same trend as the 48 hr analysis. Chlorophyll a is virtually absent from all substrates except those in the controls, and P.I's are generally very low.

The general decline in chlorophyll a and P.I. of the controls as the experiment progressed was probably caused by the change in analog operation from continuous flow (during the aufwuchs growth period) to a static system during the hydrazine spill period. However, the much larger chlorophyll a content and P.I. in the controls relative to the analogs containing hydrazine indicates that hydrazine was responsible for the aufwuchs die-off.

Table 17

Biomass of Standing Crop - Hydrazine Bioassay

| Analog Symbol | Standing Crop (Mean ± s) | | | | | |
|---|---|---|---|---|---|---|
| | Before H Added | | After 48-hr | | After 96-hr | |
| | Dry Weight | | Dry Weight | | Dry Weight | |
| | mg/Auf | % Organic | mg/Auf | % Organic | mg/Auf | % Organic |
| A | 23.8 ± 4.2 | 37.5 ± 1.8 | 27.0 ± 5.0 | 28.2 ± 5.4 | 45.4 ± 14.2 | 29.7 ± 7.7 |
| E | 19.0 ± 3.5 | 39.8 ± 7.3 | 21.0 ± 5.6 | 20.9 ± 10.0 | 46.6 ± 19.2 | 35.2 ± 10.5 |
| D | 28.4 ± 5.1 | 36.4 ± 14.8 | 27.2 ± 6.2 | 22.4 ± 5.0 | 39.3 ± 15.4 | 26.1 ± 16.0 |
| B | 28.6 ± 5.8 | 29.3 ± 12.7 | 18.5 ± 3.5 | 17.5 ± 4.0 | 34.5 ± 18.3 | 25.0 ± 9.5 |
| G | 28.7 ± 9.7 | 39.9 ± 10.7 | 17.6 ± 5.6 | 28.9 ± 23.7 | 42.7 ± 8.5 | 19.1 ± 4.5 |
| C | 44.9 ± 12.2 | 31.6 ± 4.9 | 26.9 ± 6.5 | 18.9 ± 4.3 | 37.0 ± 19.6 | 23.0 ± 8.8 |
| F | 19.2 ± 5.1 | 38.3 ± 12.2 | 18.5 ± 2.9 | 22.1 ± 4.7 | 47.5 ± 12.1 | 19.7 ± 7.6 |

Table 18

Effect of Hydrazine Spill on Aufwuchs

| Analog Symbol | Hydrazine (H) concentration, mg/ℓ | | | Chlorophyll a mg/g dry wt. Aufwuchs | | | Photosynthetic Index, mg $O_2$/g dry wt./hr. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial | After 48-hr | After 96-hr | Before H Added | After 48-hr | After 96-hr | Before H Added | After 48-hr | After 96-hr |
| A | 0 | 0 | 0.2 | 1.6 | 1.0 | 0.31 | 8.4 | 6.5 | 2.9 |
| E | 0 | 0 | 0 | 2.1 | 0.90 | 0.35 | 11.8 | 7.9 | 3.7 |
| D | 3.2 | 1.8 | 1.1 | 1.5 | 0.64 | 0.07 | 6.2 | 0.74 | 0 |
| B | 5.6 | 4.1 | 2.9 | 2.2 | 0.89 | 0.06 | 10.9 | 0 | 0 |
| G | 5.6 | 4.0 | 2.9 | 1.6 | 0.75 | 0.03 | 9.1 | 0 | 0 |
| C | 10.0 | 7.6 | 6.2 | 1.7 | 0.76 | 0.05 | 5.6 | 0 | 0 |
| F | 10.0* | 0.8* | 0.6* | 2.0 | 0.86 | 0.13 | 11.2 | 5.4 | 0.74 |

* See text - leaky partition—diffusion with adjoining tank (fish rearing tank)

## Discussion

Aufwuchs appear to be far more sensitive to hydrazine than sticklebacks. Based on the present study we conclude that the acute toxicity of hydrazine in a spill situation is below an initial level of 3.2 mg/ℓ.

The defective Tank F provided useful information because it showed that a high initial hydrazine concentration of 10 mg/ℓ, if quickly removed, may not be as environmentally damaging as prolonged exposure to much lower (0.5 to 1 mg/ℓ) hydrazine concentrations.

Appendix A

Analytical Precision of GC Determination of JP-8

| Day | JP-8 | |
|---|---|---|
| | mg/ℓ | mean ± s |
| 1 | 10.8, 10.4 | 10.60 ± 0.28 |
| 3 | 11.7, 10.7 | 11.20 ± 0.71 |
| 6 | 10.8, 10.1 | 10.45 ± 0.49 |
| 7 | 9.8, 10.5 | 10.15 ± 0.49 |
| 8 | 9.9, 10.1 | 10.00 ± 0.14 |
| 13 | 10.7, 10.7 | 10.70 ± 0.00 |
| 15 | 10.7, 10.7 | 10.70 ± 0.00 |
| 16 | 9.8, 10.7 | 10.25 ± 0.64 |
| 17 | 11.0, 11.1 | 11.05 ± 0.07 |
| 20 | 11.1, 10.7 | 10.90 ± 0.28 |
| 21 | 11.7, 11.0 | 11.35 ± 0.49 |
| 23 | 9.6, 9.6 | 9.60 ± 0.00 |
| 24 | 10.1, 9.6 | 9.85 ± 0.35 |
| 25 | 10.2, 10.8 | 10.60 ± 0.42 |
| 27 | 12.6, 12.6 | 12.60 ± 0.00 |
| 28 | 12.5, 13.2 | 12.85 ± 0.49 |
| 29 | 11.4, 11.6 | 11.50 ± 0.14 |
| 32 | 11.4, 11.3 | 11.35 ± 0.07 |
| 33 | 11.3, 10.8 | 11.05 ± 0.35 |
| 34 | 10.4, 10.1 | 10.25 ± 0.21 |
| 40 | 10.2, 10.7 | 10.45 ± 0.35 |
| 41 | 9.8, 10.7 | 10.25 ± 0.64 |
| 46 | 9.8, 9.6 | 9.70 ± 0.14 |
| 47 | 9.9, 9.8 | 9.85 ± 0.07 |
| 49 | 8.7, 9.2 | 8.95 ± 0.35 |
| 53 | 11.0, 11.3 | 11.15 ± 0.21 |
| 54 | 11.0, 10.5 | 10.75 ± 0.35 |
| 55 | 12.3, 12.5 | 12.40 ± 0.14 |
| 56 | 12.0, 10.7 | 11.35 ± 0.92 |
| 57 | 11.1, 11.1 | 11.10 ± 0.00 |
| 60 | 13.1, 13.4 | 13.25 ± 0.21 |

Appendix A (Continued)

| Day | JP-8 | |
|---|---|---|
| | mg/ℓ | mean ± s |
| 61 | 13.2, 12.2 | 12.70 ± 0.71 |
| 62 | 10.8, 10.5 | 10.65 ± 0.21 |
| 63 | 11.1, 10.4 | 10.75 ± 0.49 |
| 67 | 9.5, 9.9 | 9.70 ± 0.28 |
| 68 | 9.9, 10.5 | 10.20 ± 0.42 |
| 69 | 10.7, 10.2 | 10.45 ± 0.35 |
| 71 | 9.9, 10.4 | 10.15 ± 0.35 |
| 76 | 10.2, 9.0 | 9.60 ± 0.85 |
| 77 | 9.9, 9.9 | 9.90 ± 0.00 |
| 82 | 10.8, 10.2 | 10.50 ± 0.42 |
| 83 | 11.1, 11.0 | 11.05 ± 0.07 |
| 84 | 10.8, 10.7 | 10.75 ± 0.07 |
| 92 | 10.7, 9.8 | 10.25 ± 0.64 |
| 96 | 13.1, 12.8 | 12.95 ± 0.21 |
| 97 | 11.7, 12.0 | 11.85 ± 0.21 |
| 98 | 11.7, 11.6 | 11.65 ± 0.07 |
| 99 | 10.8, 10.7 | 10.75 ± 0.07 |
| 102 | 10.8, 10.7 | 10.75 ± 0.07 |
| 106 | 10.6, 10.8 | 10.70 ± 0.14 |
| 111 | 10.4, 10.1 | 10.25 ± 0.21 |
| 112 | 9.9, 10.2 | 10.05 ± 0.21 |
| 116 | 11.4, 11.9 | 11.65 ± 0.35 |
| 118 | 10.7, 10.7 | 10.70 ± 0.00 |
| 120 | 12.3, 12.5 | 12.40 ± 0.14 |
| 124 | 11.1, 10.8 | 10.95 ± 0.21 |
| 127 | 11.1, 12.3 | 11.70 ± 0.85 |
| 131 | 11.0, 11.4 | 11.20 ± 0.28 |
| 132 | 11.6, 11.9 | 11.75 ± 0.21 |
| 133 | 11.9, 10.7 | 11.30 ± 0.85 |
| 134 | 12.8, 12.2 | 12.50 ± 0.42 |
| 137 | 11.6, 11.4 | 11.50 ± 0.14 |

| Day | JP-8 | |
|---|---|---|
| | mg/ℓ | mean ± s |
| 138 | 11.7, 11.0 | 11.35 ± 0.49 |
| 140 | 11.4, 11.1 | 11.25 ± 0.21 |
| 141 | 12.9, 11.6 | 12.25 ± 0.92 |
| 144 | 10.4, 11.0 | 10.70 ± 0.42 |
| 147 | 10.1, 9.9 | 10.00 ± 0.14 |
| 148 | 13.4, 13.3 | 13.35 ± 0.07 |
| 152 | 11.1, 11.6 | 11.35 ± 0.35 |

n = 69 pairs, mean = 10.98 ± 0.94, s of s = 0.24

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